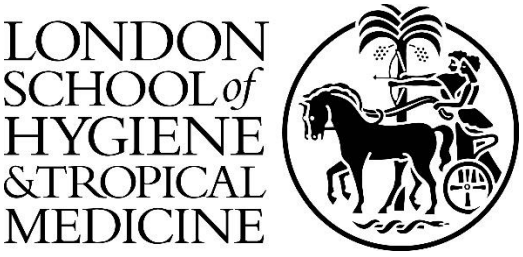


# Intermittent preventive treatment of malaria in pregnancy and infectious causes of adverse birth outcomes in sub-Saharan Africa

**RAYMUND MATTHEW CHICO**

***Submitted in accordance***
***with requirements for the degree of***
***Doctor of Philosophy***

**Department of Disease Control**
**Faculty of Infectious and Tropical Diseases**
**London School of Hygiene and Tropical Medicine**
**University of London**

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# Declaration

I, Raymund Matthew Chico, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature:  Date: 5 January 2018

| List of abbreviations | |
|---|---|
| ANC | Antenatal care |
| BPG | Benzathine penicillin G |
| CI | Confidence interval |
| *Pfdhfr* | *Plasmodium falciparum* dihydrofolate reductase |
| *Pfdhps* | *Plasmodium falciparum* dihydropteroate synthase |
| HIV | Human immunodeficiency virus |
| IPTp | Intermittent preventive treatment of malaria in pregnancy |
| ITN | Insecticide treated net |
| GRADE | Grading of Evidence, Assessment, Development and Evaluation |
| LBW | Low birth weight |
| MAP | Malaria Atlas Project |
| MIC | Minimum inhibitory concentration |
| OR | Odds ratio |
| PCR | Polymerase chain reaction |
| *Pf*$PR_{2-10}$ | *Plasmodium falciparum* prevalence rate amongst 2-10 year olds |
| PRISMA | Preferred Reporting Items for Systematic Reviews and Meta-Analyses |
| RCT | Randomised controlled trial |
| RPR | Rapid plasma reagin |
| RR | Risk ratio |
| RTI | Reproductive tract infection |
| SP | Sulphdoxine-pyrimethamine |
| STI | Sexually transmitted infection |
| STIs/RTIs | Sexually transmitted and reproductive tract infections |
| TPHA | *Treponema pallidum* haemagglutination assay |
| TRUST | Toluidine red unheated serum test |
| WHO | World Health Organization |

## Abstract

**Background**

The World Health Organization recommends intermittent preventive treatment of malaria in pregnancy (IPTp) using sulphadoxine-pyrimethamine (SP) during antenatal visits in moderate to high transmission areas. In some areas of Africa, recent efforts to control and eliminate malaria have yielded historic reductions in transmission intensity that have occurred alongside concomitant increases in parasite resistance to SP, compromising the efficacy of IPTp. Nevertheless, IPTp-SP continues to have beneficial effect on birth outcomes, and there is a suspicion that SP may protect against adverse birth outcomes attributable to curable sexually transmitted and reproductive tract infections (STIs/RTIs). This doctoral thesis explores five research questions related to IPTp with methods noted in parentheses.

**Research questions and methods**

1. In the context of declining malaria transmission, is there a threshold of malaria transmission intensity below which IPTp-SP may no longer protect against the incidence of low birth weight? (Methods: systematic review, meta-analysis, and meta-regression analysis)
2. In the context of declining parasite sensitivity to SP, is there a threshold of the *Plasmodium falciparum* resistance to SP defined by the prevalence of *dhps* mutation at codon A581G above which IPTp-SP may no longer protect against the incidence of low birth weight? (Methods: systematic review and meta-analysis)
3. In the context of declining malaria transmission and parasite sensitivity to SP, might protection conferred by IPTp-SP be explained partially by an effect against malaria infection as well as STIs/RTIs? (Methods: descriptive analysis and multivariate logistic regression)

4. In the context of pregnant women attending antenatal care in sub-Saharan Africa, what is the prevalence of malaria infection and curable STIs/RTIs? (Methods: systematic review and meta-analysis)
5. In the context of a high dual burden of malaria infection and curable STIs/RTIs amongst pregnant women in sub-Saharan Africa, would azithromycin be an efficacious drug to be included as part of IPTp? (Methods: systematic review and selected meta-analysis)

**Results**

Evidence suggests that IPTp-SP protects against low birth weight in all gravidae regardless of transmission intensity. This protection persists among primi- and secundigravidae irrespective of the prevalence of the A581G mutation. Protection appears to wane, however, as there is no evidence of protective effect against low birth weight
amongst multigravidae where the prevalence of A581G is >10.1%. Despite this finding, data from Zambia suggests that the protective effect of IPTp-SP may safeguard pregnancies against more than just the effects of malaria infection; women who received more doses of IPTp-SP during pregnancy were protected against adverse birth outcomes attributable to co-infection with malaria and several curable STIs/RTIs. Meta-analysis of data from pregnant women attending antenatal care facilities in sub-Saharan Africa suggests that malaria infection and curable STIs/RTIs amongst pregnant women attending antenatal care facilities in sub-Saharan Africa is very high and, when considered collectively, curable STIs/RTIs may be more prevalent than malaria infection during pregnancy. A potential response to this dual burden of disease in pregnancy is to explore combination therapies that address malaria and curable STIs/RTIs jointly and more effectively than IPTp-SP. Research presented in this thesis suggests that curable

STIs/RTIs are sensitive to azithromycin and that policymakers need additional evidence to consider adding azithromycin to IPTp regimens.

**Conclusions**

Despite evidence of parasite resistance, IPTp-SP remains protective against the effects of malaria infection in most pregnant women, even where transmission intensities are very low, and may also reduce the burden of curable STIs/RTIs. However, this protection is likely sub-optimal and, given the high prevalence of malaria and curable STIs/RTIs among pregnant women in sub-Saharan Africa, alternative therapies that include azithromycin merit investigation in clinical trials with robust microbiological components.

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For anyone who knows Professor Daniel Chandramohan, my supervisor and line manager at the London School of Hygiene & Tropical Medicine, it is not a surprise to hear that he has had a profound influence on me as student and faculty member. Daniel has nurtured my scientific curiosity and has shown me that sound research requires a commitment to following the path of evidence. He taught me to present my findings dispassionately and to remain open to alternative interpretations. Daniel is patient and knows that insights can take time incubate.

I also want to acknowledge my advisor Sir Professor Brian Greenwood. Despite facing perpetual demands for his time and expertise, Brian has always been generous with substantive guidance on proposals, protocols, and manuscripts. He routinely pushed me to consider the broader policy implications of my research and facilitated an opportunity for me to present preliminary results from my analysis of malaria transmission intensity and IPTp-SP protection to the Evidence Review Group on Malaria in Pregnancy hosted by the World Health Organization in Geneva in July of 2013. I was fortunate to have been invited back in July of 2017 to the same Evidence Review Group to present four published papers of mine as listed on pages 16-19. I am also grateful to Cono Ariti who has provided me with steadfast guidance on a range of statistical matters. His passion for data precision rubbed off on me, as did his penchant for ANZAC biscuits at Store Street Expresso. Enesia Banda Chaponda kindly allowed me to use data from Zambia to explore the protection conferred by sulphadoxine-pyrimethamine against adverse birth outcomes associated with malaria and curable sexually transmitted and reproductive tract infections. I have received advice and support from other colleagues including Professors Philippe Mayaud, David Mabey, Cally Roper, Carine Ronsmans, Jorge Cano Ortega, Tim Collier, and Rudiger Pittrof. The Contributions section on pages 16 to 19 provides further detail.

Laura Karr Shipler Chico, my wife, has been unequivocally supportive of this journey, something that I do not take for granted. She has been my anchor and companion for over 20 years, through spring and neap tides. Along the way, she has acquired an impressive knowledge of malariology, enough so that in social settings she is often the one who can more succinctly explain my research! She is rock solid with core beliefs that have been a ballast for me through the stormiest seas. Long ago, she introduced me to the poem *Ithaca*, an apt metaphor, I hope, for my approach to research and, well, this life. Benjamin Karr Chico and Dylan Robinson Chico, my two wonderful sons, were born during this journey, in 2007 and 2011, respectively. They have been a constant source of joy and balance in my life. I have many wishes for them and their years ahead, not the least of which is that they harbour a keen curiosity about the world and a desire to make it a better place.

## Ithaca

When you start on your journey to Ithaca,
then pray that the road is long,
full of adventure, full of knowledge.
Do not fear the Lestrygonians
And the Cyclopes and the angry Poseidon.
You will never meet such as these on your path,
if your thoughts remain lofty, if a fine
emotion touches your body and your spirit.
You will never meet the Lestrygonians
And the Cyclopes and the fierce Poseidon,
if you do not carry them within your soul,
if your soul does not raise them up before you.

Then pray that the road is long,
That the summer mornings are many,
That you will enter ports seen for the first time
With such pleasure, with such joy!
Stop at the Phoenician markets,
and purchase fine merchandise,
mother-of-pearl and corals, amber and ebony,
and pleasurable perfumes of all kinds,
buy as many pleasurable perfumes as you can;
visit hosts of Egyptian cities,
to learn and learn from those who have knowledge.

Always keep Ithaca fixed in your mind.
To arrive there is your ultimate goal.
But do not hurry the voyage at all.
It is better to let it last for long years;
and even to anchor at the isle when you are old,
rich with all that you have gained on the way,
not expecting that Ithaca will offer you riches.

Ithaca has given you the beautiful voyage.
Without her you would never have taken the road.
But she has nothing more to give you.

And if you find her poor, Ithaca has not defrauded you.
With the great wisdom you have gained, with so much experience,
you must surely have understood by then what Ithaca means.

**Constantine P. Cavafy (1911)**

## Contributions

In preparing this doctoral thesis, I have drawn from several of the peer-reviewed papers that I have published as first and corresponding author during my tenure at the London School of Hygiene & Tropical Medicine. Where applicable, I have re-written text from these publications to present a single monograph that addresses five inter-connected research questions. The matrix below summarises the sections in this thesis where I have adapted content from these publications and presentations along with a brief summary of contributions made by others. Note that I recently presented four of these papers to the World Health Organization's Evidence Review Group on Intermittent Preventive Treatment of Malaria in Pregnancy between 12 and 14 July 2017 in Geneva, Switerland.

| Section | Publication | Presentation | Contribution |
|---|---|---|---|
| Literature review | **Chico RM**, Pittrof R, Greenwood B, Chandramohan D. Azithromycin-chloroquine and the intermittent preventive treatment of malaria in pregnancy. *Malaria Journal*. 2008;7(1):255.[1] | NA | Rudi Pittrof offered me comments on the manuscript, as did Professor Brian Greenwood. Professor Daniel Chandramohan provided guidance on my analysis and interpretation. |
| | **Chico RM,** Chandramohan D. Azithromycin plus chloroquine: combination therapy for protection against malaria and sexually transmitted infections in pregnancy. *Expert Opinion on Drug Metabolism & Toxicology* 2011; **7**(9): 1153-67.[2] | **Chico RM** presented this paper to the World Health Organization's Evidence Review Group on Intermittent Preventive Treatment (IPT) of malaria in pregnancy (12-14 July 2017). Geneva, Switerland. | Professor Daniel Chandramohan provided guidance on my analysis and interpretation. |

| Section | Publication | Presentation | Contribution |
|---|---|---|---|
| Literature review | **Chico RM** and Chandramohan D, Intermittent preventive treatment of malaria in pregnancy: at the crossroads of public health policy. *Tropical Medicine and International Health*, 2011;16(7): p. 774–785.[3] | NA | Professor Daniel Chandramohan provided guidance on my analysis and interpretation. |
| Q 1 | **Chico RM**, Cano J, Ariti C, Collier TJ, Chandramohan D, Roper C, et al. Influence of malaria transmission intensity and the 581G mutation on the efficacy of intermittent preventive treatment in pregnancy: systematic review and meta-analysis. *Tropical Medicine and International Health*. 2015;20(12):1621-33.[4] | **Chico RM** presented preliminary findings to the World Health Organization's Evidence Review Group on Intermittent Preventive Treatment (IPT) of malaria in pregnancy (11-13 July 2013). Geneva, Switerland.[5]<br><br>**Chico RM** presented refined results at the 63rd annual meeting of the American Society of Tropical Medicine and Hygiene (2-6 November 2014). New Orleans, USA.[6] | Jorge Cano offered assistance in the use of raster data from the Malaria Atlas Project; Cono Ariti and Timothy Collier assisted me with data analyses; Professors Daniel Chandramohan and Brian Greenwood guided me in study design, analysis and interpretation of results. |
| Q 2 | **Chico RM**, Cano J, Ariti C, Collier TJ, Chandramohan D, Roper C, et al. Influence of malaria transmission intensity and the 581G mutation on the efficacy of intermittent preventive treatment in pregnancy: systematic review and meta-analysis. *Tropical Medicine and International Health*. 2015;20(12):1621-33.[4] | NA | Professor Cally Roper provided me with prevalence data specific to the 581G mutation. |

| Section | Publication | Presentation | Contribution |
|---|---|---|---|
| Q 3 | **Chico RM**, Chaponda EB, Ariti C, Chandramohan D, Sulfadoxine-pyrimethamine exhibits dose-response protection against malaria and sexually transmitted and reproductive tract infections and related adverse birth outcomes. *Clinical Infectious Diseases.*2017;64(8):1043-1051.[7] | **Chico RM** presented preliminary results at the 65rd annual meeting of the American Society of Tropical Medicine and Hygiene (13-17 November 2016). Atlanta, USA.[8]<br><br>**Chico RM** presented this paper to the World Health Organization's Evidence Review Group on Intermittent Preventive Treatment (IPT) of malaria in pregnancy (12-14 July 2017). Geneva, Switerland. | Enesia Chaponda provided me data from Zambia for analyses; Cono Ariti assisted me with data analysis. Professor Daniel Chandramohan assisted me with my study design, analysis and interpretation. |
| Q 4 | **Chico RM**, Mayaud P, Ariti C, Mabey D, Ronsmans C, Chandramohan D. Prevalence of malaria and sexually transmitted and reproductive tract infections in pregnancy in sub-Saharan Africa: A systematic review. JAMA: *Journal of the American Medical Association*. 2012;307(19):2079-86.[9]<br><br>*Results have been re-published in:*<br>Mabey D, Gill G, Parry E, Weber MW & Whitty CJM. *Principles of Medicine in Africa*. 4th edition (Cambridge University Press, 2013).[10] | **Chico RM** presented results as part of a seminar sponsored by Maternal Adolescent Reproductive and Child Health Centre. London School of Hygiene & Tropical Medicine. (18 June 2012) London, UK.[11]<br><br>**Chico RM** presented this paper to the World Health Organization's Evidence Review Group on Intermittent Preventive Treatment (IPT) of malaria in pregnancy (12-14 July 2017). Geneva, Switerland. | Professors Philippe Mayaud and David Mabey provided me with guidance specific to curable sexually transmitted and reproductive tract infections; Professor Carine Ronsmans offered helpful comments and suggestions specific to maternal health and systematic reviews; Cono Ariti assisted me with data analysis. Professor Daniel Chandramohan assisted me with my study design, analysis and interpretation. |

| Section | Publication | Presentation | Contribution |
|---|---|---|---|
| Q 5 | **Chico RM**, Hack BB, Newport MJ, Ngulube E, Chandramohan D. On the pathway to better birth outcomes? A systematic review of azithromycin and curable sexually transmitted infections. *Expert Reviews on Anti-Infective Therapy*. 2013;11(12):1303-32.[12] | **Chico RM** presented this paper to the World Health Organization's Evidence Review Group on Intermittent Preventive Treatment (IPT) of malaria in pregnancy (12-14 July 2017). Geneva, Switerland. | Birken Hack contributed to the document review; Professor Melanie Newport offered comments on the manuscript; Enesia Ngulube provided me data on the effect of curable STIs/RTIs on pregnancy outcomes. Professor Daniel Chandramohan assisted me with analysis and interpretation. |
| Discussion | **Chico RM** and Moss WJ. Prevention of malaria in pregnancy: a fork in the road? *The Lancet*. 2015.[13] | NA | Professors Chandramohan and Greenwood reviewed the first draft. Professor Moss provided helpful comments and suggestions. |

# Introduction

Some 2,400 years ago in ancient Greece, Hippocrates observed that pregnant women were vulnerable to life-threatening fevers.[14] The extent to which these fevers were attributable to malaria is unknown since the aetiology of malaria infection was poorly understood until 1880 when Alponse Laveran, aided by crude microscopy, first observed *Plasmodium falciparum* in the blood-film slides of febrile patients under his care in Algeria.[15] Two years later he reported that women who experienced symptoms associated with *P. falciparum* infection had higher placental parasite densities than mothers who had been free of malarial symptoms throughout their pregnancies.[16] In sub-Saharan Africa, one of the earliest published descriptions of the burden of malaria infection during pregnancy comes from D.B. Blacklock and R.M. Gordon who estimated in 1925 that women in Sierra Leone with placental parasitaemia at delivery were five times more likely to have their newborn die than mothers whose placentas had not been parasitised.[17] Steketee *et al.* calculated that each year 75,000 to 200,000 infant deaths in sub-Saharan Africa are attributable to malaria infection during pregnancy, whereas Murphy and Breman estimated that malaria-induced low birth weight (LBW) kills 62,000 to 363,000 newborns each year in the region.[18,19] This mortality is largely attributable to the effect of malaria infection during pregnancy on low birth weight (LBW) associated with preterm birth (population attributable risk: 8% to 36%) and LBW associated with intrauterine growth retardation (population attributable risk: 13% to 70%).[18]

To safeguard pregnant women against the consequences of malaria infection during pregnancy who are resident in areas of moderate (stable) to high transmission, the

World Health Organization (WHO) recommends the provision of intermittent preventive treatment in pregnancy (IPTp) using sulphadoxine-pyrimethamine (SP) at every scheduled antenatal care (ANC) visit from the second trimester until delivery.[20] Meta-analysis suggests that any chemoprophylaxis reduces the incidence of LBW amongst primi- and secundigravidae by 27% (Relative Risk [RR] = 0.73; 95% Confidence Intervals [CI]: 0.61, 0.87; 8 trials; N = 3,619), severe maternal anaemia by 40% (RR 0.60, 95% CI: 0.47, 0.75; 3 trials, N = 2,503), and neonatal mortality 38% (RR = 0.62; 95% CI: 0.37, 1.05; 2 trials; N = 2,156).[21] The intervention also protects against asymptomatic parasitaemia, peripheral and placental infection that may be present at the time of dosing while, separately, shielding against new malaria infections that may occur after dosing and before the next ANC visit.[22] The parasiticidal effect of SP against *P. falciparum* relies on pharmacological synergy between both drug components.[23] Sulphadoxine inhibits dihydropteroate synthase (*Pfdhps*), an enzyme required by *P. falciparum* to biosynthesize folate, whereas pyrimethamine inhibits dihydrofolate reductase (*Pfdhfr*), an essential enzyme used by *P. falciparum* to produce tetrahydrofolate, a cofactor the parasite needs to biosynthesise DNA and protein.[24,25] Several parasite mutations are associated with decreased SP sensitivity that vary geographically by type and prevalence.[26] In West Africa, 'partially resistant' biomarkers are found in combinations of *Pfdhfr* N51I, N59R, and S108N with *Pfdhps* A437G, whereas parasites in East Africa express 'fully resistant' combinations of *Pfdhfr* N51I, N59R, and S108N with *Pfdhps* A437G and K540E.[26] Epigenetic analyses suggest that there are three foci in East Africa where parasite populations have acquired the additional mutation of *Pfdhps* A581G to become 'super resistant' to SP.[26]

There have been historic reductions in malaria transmission intensity in some locales of sub-Saharan Africa over the past decade, accelerated by malaria control interventions that have included the use of indoor residual spraying, artemisinin-combination therapies, and mass distribution of insecticide treated nets (ITNs). Of the 35 sub-Saharan countries where IPTp-SP is national policy, ITNs have been made available to pregnant women at no direct cost in 33 countries.[27] This has resulted in an estimated 10.5 million women using ITNs during their pregnancies in 2010 across 37 sub-Saharan countries, representing 38.8% of all births in the region (95% CI: 34.6%, 43.0%).[5] The concurrent increase in SP drug resistance and decline in malaria transmission intensity in some settings has raised competing questions of where and when IPTp-SP should be replaced with a more efficacious antimalarial drug – or where and when the intervention should be discontinued all together. This doctoral thesis addresses these and other related questions.

# Literature review

## Epidemiology of malaria in pregnancy

Malaria infection during pregnancy is associated with a variety of adverse birth outcomes including maternal anaemia,[28-31] stillbirth,[32-34] intrauterine growth retardation,[35,36] preterm birth, [36,37] and LBW,[32,35,37-39] and infant mortality.[39] Amongst these adverse birth outcomes, LBW is the most frequently reported outcome because of its ease and precision of measurement. Arvo Ylppö, the acclaimed Finnish paediatrician and early advocate for child welfare clinics, defined LBW as newborns weighing less than 2.5 kg at delivery.[40] A landmark cohort study in the 1980s showed that LBW infants in low-income settings were 4.3 times more likely to die during the neonatal period than newborns who were delivered weighing 2.5 kg or more.[41] Low-birth weight babies who survive infancy then go on to experience higher rates of respiratory infection and diarrhoeal disease than peers who were born above the 2.5 kg threshold[42,43] and, when fully grown, are more likely to suffer from micro-vascular conditions.[44-46] Low birth weight girls, as adults, are at greater risk of developing pre-eclampsia and delivering LBW babies themselves.[47] Thus, reducing the incidence of LBW has public health implications that are lifelong and intergenerational.

### Entomological considerations

Female *Anopheles* mosquitoes are the primary vectors of malaria parasites. While in the process of collecting blood meals for egg development, infected *Anopheles* inject *P. falciparum* sporozoites into humans. *Anopheles* are more likely to target expecting mothers compared to non-pregnant women for several reasons. Female *Anopheles* are attracted to the carbon dioxide that humans exhale,[48] with and without any

accompanying human scent.[49] Although the extent to which emitted carbon dioxide and temperature may vary over the course of pregnancy, researchers in the Gambia found that expecting mothers with a mean gestational age of 28 weeks had 21% more expiratory lung volume and abdomens that were also 0.7$^{o}$ Celsius warmer compared to non-pregnant women.[50] A separate study, also in The Gambia, showed the extent to which pregnant women attract more *A. gambiae*, the most common malaria vectors in sub-Saharan Africa. In total, 72 women, 36 pregnant and 36 non-pregnant, were given malaria chemoprophylaxis and bed-nets prior to sleeping in identical experimental huts. Over three consecutive nights, twice as many *Anopheles* entered the huts of pregnant participants compared to non-pregnant women ($P < 0.000$). Adding to the risk of malaria exposure, pregnant women were observed stepping out of their protective nets an average of twice per night versus once amongst non-pregnant participants ($P = 0.019$).[51]

**Physiological considerations**

In addition to being at elevated risk of exposure, pregnant women are at greater risk of adverse consequences resulting from malaria infection compared to non-pregnant women. During pregnancy, maternal inflammatory responses are down-regulated for the benefit of embryonic implantation and foetal survival.[52] Hence, pregnant women may be more vulnerable to new infections, relapsed infections (from latent or dormant phases), recrudescent infections (delayed clearance of infection), and re-activated infections (from persistent chronic infections).[53] Maternal parasitaemia triggers inflammatory responses that increase circulation of tumour necrosis factor alpha (TNF-α) and interleukin-10[54] which are independently associated with adverse

birth outcomes.[55] Chronic parasitaemia is associated with intrauterine growth retardation, while acute infection is more commonly the cause of miscarriage or stillbirth.[56] Although placental malaria does not appear to interfere with the transport of vitamin A,[57] iron,[58] folic acid or vitamin B-12,[53] general placental insufficiency may compromise the exchange of other vital nutrients required for healthy foetal development.[59,60] In addition, parasitic invasion of the trophoblast may have an effect similar to pre-eclampsia, decreasing placental circulation by undermining normal remodelling of spiral arteries into utero-placental vessels.[61] The risk of maternal hypertension may increase amongst primigravidae exposed to chronic placental malaria, potentially triggered by a foetal response to placental inflammation.[62]

Amongst the five malaria parasites known to infect humans, *P. falciparum* is unique. Erythrocytes penetrated by *P. falciparum* in pregnant women will commonly adhere to chondroitin sulphate A (CSA) molecules and sequester along the endothelial and syncytiotrophoblast cells of the placenta.[63] As a consequence, infected erythrocytes may not be detected with conventional assays that rely on samples of peripheral blood.[64] Even still, malaria infection does not affect all pregnant women equally. Parasites that bind to CSA molecules also transcribe the var 2 CSA gene,[65-67] triggering development of protective immunoglobulin G that is associated with maternal parity in malaria-endemic areas.[68] Thus, multigravidae exposed to CSA-binding parasites during previous pregnancies are better able to control parasite densities than primi- and secundigravidae. Maternal infection with the human immunodeficiency virus (HIV) compromises the acquisition of semi-immunity against malaria infection during pregnancy.[63]

## Chemoprevention

### Chloroquine and pyrimethamine monotherapies

The WHO first recommended the provision of chemoprophylaxis to all pregnant women living in malaria-endemic areas in 1986. This involved weekly, self-administered doses of 300 mg chloroquine or 25 mg pyrimethamine.[69] Compliance with these regimens proved difficult,[70,71] and the spread of *P. falciparum* resistance to chloroquine and, separately, to pyrimethamine undermined the protective effect of both regimens.[70-72]

### Sulphadoxine-pyrimethamine

In 1993, Malawi became the first country to adopt a national strategy of directly-observed chemoprevention using SP administered to pregnant women during ANC visits.[73] This operational experience, coupled with clinical trial data from Kenya[74,75] and Malawi,[38,76] was sufficient for the WHO to make its first IPTp-SP policy recommendation.[22] At present, 35 of the 36 countries worldwide that have an IPTp-SP policy are in sub-Saharan Africa.[27]

## Decreasing malaria transmission

Over the past decade, as ITN provision has become part of the standard of care, nets have been used in IPTp-SP trials. This has greatly affected trial outcomes and the transmission intensity to which participants have been exposed. A randomised placebo-controlled trial in Mozambique conducted between 2003 and 2005 (N = 1,030) amongst women of all gravidae, 90% of whom used ITNs, showed that two doses of IPTp-SP had no protective effect against LBW (Relative Risk [RR], 0.99; 95% CI: 0.70, 1.39), nor against placental infection (RR = 1.0; 95% CI: 0.88, 1.13; *P* = 0.964)

when compared to placebo. It is not clear the extent to which this observation was attributable to lost parasite sensitivity to SP, or to women having been exposed less malaria infection, or both. Despite these findings, IPTp-SP conferred some important benefits. Women who received SP versus no doses had 40% fewer clinical malaria cases (95% CI: 7.4%, 61.2%]; *P* = 0.02), and one-half the prevalence of peripheral parasitaemia (7.10% versus 15.15%) (*P* = 0.001). Moreover, compared to ITNs alone, IPTp-SP provided greater protection against parasitaemia (RR = 0.52; 95% CI: 0.27, 0.99; *P* = 0.044) in the first eight weeks post-partum,[77] representing an important chemoprophylactic effect because the maternal risk of malaria infection has been shown to increase following delivery into the early post-partum period.[78] Specifically, the risk of malaria infection is three times greater in the second (adjusted risk ratio = 2.8; range 1.1 to 7.4; *P* = 0.04) and third trimesters (adjusted risk ratio = 3.1; range 1.2 to 7.9; *P* = 0.02) compared to the first trimester, and is four times greater in the 60-day post-partum period (adjusted risk ratio = 4.1; range 1.8 to 9.5; *P* = 0.001).[78] Secondary analysis of data from a Mozambique trial noted above found that IPTp-SP reduced early neonatal ( $\leq$ 7 days of life) mortality by 61.3% (95% CI: 7.4%, 83.8%); *P* = 0.024] (20 deaths overall; 15 in the placebo group versus five in the IPTp-SP group; *P* = 0.039).[79] Protection was also reported against neonatal ( $\leq$ 28 days of life) mortality (25 deaths overall; 18 in the placebo group versus seven in the IPTp-SP group; *P* = 0.041), a trend that was no longer statistically significant against infant (first 12 months of life) mortality (58 deaths overall; 35 in the placebo group versus 23 in the IPTp-SP group; *P* = 0.136).[79]

Another placebo-controlled trial amongst pregnant multigravidae (N = 1,035) conducted in The Gambia between 2002 and 2004 showed that IPTp-SP offered no

greater protection against LBW than ITNs alone (effect difference = 28 g; 95% CI: 11, 67; *P* = 0.16); 78% of women in both groups reported using ITNs.[80] Researchers reported no protective effect of IPTp-SP in an un-blinded randomised trial carried out from 2004 to 2007 amongst women of all gravidae (N = 5,775) in a low-transmission area of Uganda. No difference in the percentage of LBW infants was seen across the three intervention groups: IPTp-SP alone (6.48%), ITNs alone (6.28%) and IPTp-SP plus ITNs (6.85%); *P* = 0.80.[81] Table 1 contains selected studies that illustrate outcomes following the provision of placebo verses IPTp-SP with and without ITNs . The first three are trials that suggest IPTp-SP may be no better than placebo in settings where ITN coverage is very high, thereby reducing exposure to malaria transmission. The fourth is an observational study in which ITN use was low and, unexpectedly, SP use appeared to increase the risk of placental parasitaemia.

## Table 1. Selected studies of placebo vs. IPTp-SP with and without ITNs

| Country [ref] | Site | Gravidae | Placebo (with ITNs) | | | SP (2 doses without ITN) | | | SP (2 doses with ITNs) | | | Malaria transmission and ITN use | Key findings |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | LBW | Peripheral | Placental | LBW | Peripheral | Placental | LBW | Peripheral | Placental | | |
| Mozambique [77] | Manhiça | Primigravidae | 20.7% (25/121) | 23.6% (30/127) | 52.3% (219/419) | - | - | - | 21.8% (29/133) | 13.5% (18/133) | 52.1% (222/426) | Perennial transmission with seasonal variation; self-reported use of ITNs was 90% in both treatment groups | SP as effective as placebo |
| | | 1-3 pregnancies | 6.7% (13/195) | 16.1% (31/193) | - | - | - | - | 10.3% (20/194) | 6.3% (12/192) | | | |
| | | ≥4 pregnancies | 11.7% (21/180) | 8.0% (14/175) | - | - | - | - | 5.4% (9/167) | 30.4% (51/168) | | | |
| The Gambia [80] | Farafenni | Secundigravidae | 6.9% (63/917) | 3.3% (34/1,035) | - | - | - | - | 5.5% (51/931) | 9.0% (91/1,010) | - | Ben-net usage including ITNs was ~ 70% | SP as effective as placebo |
| | | Multigravidae | | | | | | | | | | | |
| Uganda [81] | Kabale | Primigravidae | 5.5% (18/329) | 12.9% (201/1,559) | 2.6% (16/622) | 5.1% (16/313) | 14.6% (231/1,580 | 4.3% (28/651) | 8.1% (27/333) | 15.3% (231/1,510) | 3.1% (19/613) | ITN coverage was estimated to be > 97% | ITNs alone, SP-IPTp alone, and ITNs plus SP-IPTp were non-inferior to each other |
| | | Secundigravidae | 5.8% (16/277) | - | - | 7.7% (22/287) | - | - | 5.1% (14/276) | - | - | | |
| | | 2 to 4 previous pregnancies | 5.9% (37/631) | - | - | 6.6% (40/610) | - | - | 7.2% (44/614) | - | - | | |
| | | ≥5 pregnancies | 8.3% (27/325) | - | - | 6.1% (21/347) | - | - | 6.7% (21/315) | - | - | | |
| Tanzania [82] | Muheza | Primigravidae | - | - | 35.3% [a] (6/17) | - | - | 45.5% [b] (35/77) | - | - | 50.0% [c] (5/10) | Perennial transmission; ITN use summarised in the table notes. | SP use increased the risk of placental parasitaemia |
| | | Secundigravidae | - | - | 41..2% [a] (7/17) | - | - | 28.6% [b] (22/77) | - | - | 40.0% [c] (4/10) | | |
| | | Multigravidae | - | - | 23.5% [a] (4/17) | - | - | 26.0% [b] 20/77) | - | - | 10.0% [c] (1/10) | | |

Notes:

[a] Women who never received IPTp-SP and 0% used ITNs

[b] Women who received 'early' IPTp-SP as noted on ANC card and 14% used ITNs

[c] Women who received 'recent' IPTp-SP as noted on ANC card and 30% used ITNs

### Decreasing parasite sensitivity

Several factors have contributed to the decline in the protective effect of IPTp-SP. Historic use of pyrimethamine monotherapy is partially responsible for the emergence of parasite resistance. The experience of Tanzania is illustrative. During the 1950s, Tanzania introduced pyrimethamine monotherapy. At the time, treatment for uncomplicated malaria infection was a single 25 mg dose which produced a clinical cure within seven days.[83] The first known treatment failure was recorded in 1953 during a clinical trial in Mingeza village, near Muheza town, north-east Tanzania.[83] Unrestricted pyrimethamine use continued in the area until 1965 when treatment failures were observed 160 kilometres from the original foci of resistance.[84] In the same year, Tanzania suspended imports of pyrimethamine, as well as progunil, an antimalarial drug that can select for pyrimethamine resistance.[85] No *Pfdhfr/Pfdhps* inhibitors were available in Tanzania for malaria chemoprevention or treatment purposes until 1980 when researchers conducted a trial in Bagamoyo, 137 kilometres south of Muheza, where there had never been any previously reported treatment failures involving pyrimethamine. In this setting, despite 15 years of suspended SP drug pressure, 47.8% (N = 23; 95% CI: 26.8%, 69.4%) of patients with uncomplicated malaria experienced recrudescent infections within seven days of pyrimethamine treatment. This suggests that the fitness-cost of mutating in response to pyrimethamine exposure is minimal and, consequently, parasite reversion to a sensitive state may not occur following the withdrawal of SP drug pressure.

Alarming evidence of SP resistance comes from a cross-sectional survey conducted in Muheza, Tanzania, between 2002 and 2005. Placental parasitaemia was significantly

higher in women given any dose of IPTp-SP compared to women who received no doses, 84% [87 of 104] versus 16% [17 of 104], respectively (*P* = 0.03). Bed-net use was lowest amongst those who received no IPTp-SP doses (23.5%) in contrast to women given treatment early in pregnancy (36%) or shortly before delivery (30%).[82,86,87] Investigators suggest that the increase in placental parasitaemia amongst women given IPTp-SP was likely the result of extreme drug resistance. The day-28 treatment failure rate was 82% amongst children under five-years of age in an area near Muheza where the parasite population exhibited near saturation of the *Pfdhfr/Pfdhps* quintuple mutation with concurrent mutation along *Pfdhps* gene at codon 581.[88] Investigators in the IPTp study postulated that parasites in locales with very high pyrimethamine resistance may be able to repopulate a human host more rapidly than parasites in the same area that remain sensitive to pyrimethamine, thereby increasing overall parasite densities amongst recipients of preventive treatment.[89,90] Despite these findings of apparent 'harm' attributable to IPTp-SP exposure, a more recent study in Malawi did not observe similar results.[91]

### Potential for azithromycin use in IPTp

Several trials over the past decade have tried to identify therapies to replace SP where parasite resistance is high. Azithromycin-based combination therapies have been amongst these candidates and may confer dual protection against malaria infection and curable STIs/RTIs.

### Azithromycin monotherapy

Synthesised in the 1980s, azithromycin is the first compound of the azalide family of antibiotics. Animal studies have shown that quantities 2 to 4 times the human daily

dose do not reduce fertility, nor cause foetal harm.[92] Pregnant women have received up to 2 g azithromycin in a single dose in all trimesters without teratogenic effect.[92] In adults, a one-time dose of 1 g azithromycin is associated with mild to moderate side-effects including diarrhoea or loose stools (7%), nausea (5%), vomiting (2%), and vaginitis (2%) with fewer than 1% of recipients experiencing dizziness, headache, vertigo, and/or somnolence.[92] Azithromycin has demonstrated better tolerability than erythromycin with shorter dosing regimens required to achieve the same therapeutic effect.[93] HIV-infected patients, however, poorly tolerate azithromycin when taken long-term as chemoprophylaxis.[94,95]

Azithromycin functions as a slow-acting macrolide that causes the progeny of exposed parasites to inherit an apicoplast that is incapable of protein synthesis, thereby producing a delayed-death in parasites.[96] This slow clinical response makes azithromycin monotherapy an unsuitable choice for treating uncomplicated or sever *P. falciparum* infections.[97] However, azithromycin monotherapy is efficacious against *P. vivax* with 1 g to 2 g[98,99] and, against *P. falciparum*, [100] has been used as a chemoprophylaxis in Kenya, Indonesia and Thailand conferring modest protection as shown in Table 2.

**Table 2. Azithromycin chemoprophylactic efficacy relative to placebo**

| Country | Treatment regimen and protective efficacy relative to placebo | | | | | | |
|---|---|---|---|---|---|---|---|
| | AZ 250 mg daily | | AZ 1 g weekly | | 750 mg loading 250 mg daily | | Notes |
| | PE% | 95% CIs | PE% | 95% CIs | PE% | 95% CIs | |
| Kenya [100] | 82.7 | 68.5, 91.1 | 64.2 | 47.1, 77.1 | - | - | *Plasmodium* species unspecified<br>59 subjects = 250 mg daily dose<br>58 subjects = 1 g weekly dose<br>10 week follow-up |
| Indonesia[98] | - | - | - | - | 71.6<br>98.9<br>84.7 | 50.3, 83.8<br>93.1, 99.9<br>75.6, 90.7 | *Plasmodium falciparum*<br>*Plasmodium vivax*<br>All malaria<br>148 subjects<br>20 week follow-up period |
| Thailand[99] | - | - | - | - | 70.8<br>98.0<br>91.8 | -14.0, 93.7<br>87.8, 99.9<br>78.6, 97.5 | *Plasmodium falciparum*<br>*Plasmodium vivax*<br>All malaria<br>179 subjects<br>20 week follow-up period |

PE = Protective efficacy; CI = Confidence interval

Not all potential partner compounds are pharmacologically compatible. An *in vitro* study suggests that azithromycin has an additive effect against *P. falciparum* when combined with either mefloquine or pyronaridine.[101] Artesunate and azithromycin, in contrast, have demonstrated antagonism when combined against fresh *P. falciparum* samples.[101,102] Antagonism, and resultant poor efficacy, may partially explain why a paediatric trial that used azithromycin plus artesunate was halted prematurely,[103] although two studies of semi-immune adults have shown favourable results.[104,105] Azithromycin could be combined with piperaquine, a quinolone compound related to chloroquine, but better tolerated.[106] The combination of azithromycin and dihydroartemisinin has shown additive-to-synergistic *in vitro* effect.[104] Only two azithromycin combination therapies have undergone investigation in clinical trials: SP plus azithromycin, and azithromycin plus chloroquine. There is burgeoning interest in combining azithromycin with dihydroartemisinin-piperaquine for use in IPTp. The European & Developing Countries Clinical Trials Partnership (EDCTP) and the Medical

Research Council in the UK are currently supporting two such trials that will begin recruitment by early 2018.

### Azithromycin plus sulphadoxine-pyrimethamine

To date, three clinical trials have compared birth outcomes following exposure to the standard of care, IPTp-SP, versus IPTp-SP plus azithromycin as summarised in Table 3. Two of these trials were conducted in Malawi and yielded contradictory results. The first was a three-arm trial between 2002 and 2006 in which pregnant women received routine IPTp-SP, or monthly IPTp-SP, or monthly IPTp-SP plus 1 g azithromycin two times during the antenatal period. The prevalence of LBW was 12.9% (52 of 402), 9.1% (36 of 394), and 7.9% (32 of 406) in women receiving IPTp-SP, monthly IPTp-SP or monthly IPTp plus azithromycin, respectively, and primi- and secundigravidae were protected more by preventive therapy against the consequences of malaria infection during pregnancy than multigravidae.[107] In contrast, the APPLe study (Azithromycin for the Prevention of Preterm Labour) in Malawi, carried out between 2004 and 2005 showed that 1 g azithromycin, administered twice during the antenatal period, had no effect on birth-weight, nor on the incidence of preterm delivery.[108] These results require cautious interpretation. There were several differences between the studies. The APPLe trial combined azithromycin with IPTp-SP given two times, whereas women in the other Malawi trial received IPTp-SP monthly, either with or without azithromycin. In addition, participants in the APPLe trial were more often primigravidae and were more likely to have syphilis at enrolment. Finally, syphilis treatment administered to women in the APPLe trial was not in accordance with WHO recommendations for pregnant women. These women were given 1g benzyl penicillin,

treatment for individuals with neurosyphilis,[109] instead of 2.4 mμ of benzathine penicillin G.[110] Moreover, treatment with azithromycin may have cleared maternal syphilis, but may not have altered birth outcomes; the compound does not perfuse the placenta in sufficient concentrations. Thus, neither the benzyl penicillin nor the azithromycin administered twice were likely to have cured congenital syphilis.[111]

A recent trial in Papua New Guinea, also summarised in Table 3, compared monthly SP plus azithromycin (1 g twice daily for 2 days; 4 g AZ total during antenatal period) against SP and chloroquine (450 to 600 mg, daily for three days) given once, followed by SP and chloroquine placebo (control). Intention-to-treat analysis showed the incidence of LBW to be 12.8% (130 of 1,013) amongst women given SP plus azithromycin and 17.4% (175 of 1,008) for women who received SP plus chloroquine. Per-protocol analysis was comparable; LBW was 12.6% (122 of 967) amongst women administered SP plus azithromycin versus 16.7% for women given SP plus chloroquine (159/952).[112]

### Azithromycin plus chloroquine

The combination of azithromycin plus chloroquine has demonstrated an additive-to-synergistic effect *in vitro* against *P. falciparum*.[101] When used against chloroquine-sensitive strains, azithromycin plus chloroquine produced an additive effect, and synergy when exposed to chloroquine-resistant *P. falciparum*. Two studies have been conducted using azithromycin plus chloroquine amongst pregnant women with a three-day regimen involving a daily dose of 1 g azithromycin and 620 mg chloroquine base (four tablets of azithromycin plus chloroquine each containing 250 mg/155 mg). A single-arm parasite clearance trial amongst 163 women with asymptomatic

infections showed the day 28 cure rate to be 99.4% (95% CI: 97.8, 100) when corrected using polymerase chain reaction (PCR) methods and intention-to-treat analysis.[113] At day 35 and day 42, PCR-corrected cure rates dropped slightly to 96.7% (95% CI: 93.4%, 99.9%), and 95.2% (95% CI: 91.3%, 99.0%), respectively. The uncorrected PCR prevalence rates at day 28, day 35, and day 42 were 95.5% (95% CI: 91.8%, 99.1%), 87.7% (95% CI: 82.1%, 93.2%), and 78.4% (95% CI: 71.6%, 85.3%), respectively.[113] A multi-centre IPTp trial compared azithromycin plus chloroquine versus SP with the primary endpoint being the proportion of sub-optimal pregnancy outcomes (LBW, premature birth, stillbirth, abortion, lost to follow-up prior to observation of pregnancy outcome, or missing birth weight) under intention-to-treat analysis. The trial was terminated early on grounds of futility; the prevalence of sub-optimal birth outcome amongst recipients of azithromycin plus chloroquine was 26.2% (95% CI: 23.9%, 28.4%) versus 23.7% (95% CI: 21.5%, 25.9%) for women given SP.[114]

## Table 3. Selected IPTp studies of azithromycin

| Country [ref] | Site | Gravidae | IPTp (standard) | | IPTp monthly | | IPTp monthly + AZ x 2 | | AZ+CQ | | Malaria transmission and ITN use | Key notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | LBW | Preterm | LBW | Preterm | LBW | Preterm | LBW | Preterm | | |
| Malawi [107] | Mangochi | 0 previous pregnancies | 12.9% 52/402 | 30.0% 33/110 | 9.1% 36/394 | 18.7% 20/107 | 7.9% 32/406 | 14.6% 13/89 | - | - | Holoendemic-transmission; self-reported use of nets previous night ranged between 59.4% and 61.0% across treatment groups (data not collected on whether nets were treated) | *T. vaginalis* was 16.8% (69/411) in IPTp (standard) group vs. 11.0% (46/419) amongst women who received IPTp monthly plus AZ x 2. |
| | | 1 previous pregnancy | | 17.4% 15/86 | | 24.4% 19/78 | | 18.8% 15/80 | - | - | | |
| | | 2 previous pregnancies | | 12.6% 30/239 | | 11.3% 29/256 | | 8.9% 24/271 | - | - | | |
| | | All gravidae | | 17.9% 78/435 | | 15.4% 68/441 | | 11.88% 52/440 | - | - | | |
| Malawi [108] | Southern | All gravidae | 2.99 kg (n=769) | 17.4% 189/1,087 | - | - | 3.03 kg (n=739) | 16.8% 184/1,096 | - | - | Malaria transmission not reported; net use not reported | AZ+SP was no more protective than SP alone, but sub-optimal Rx was given to women with syphilis in both groups. |
| Papua New Guinea [112] | Madang Province | All gravidae | 17.4%[2] 175/1,008 | - | - | - | 12.8% 130/1,013 | RR = 0.62 95% CI: 0.43,0.89, *P* = 0.010 | - | - | Women without nets were given an ITN; due to local stock-outs 8% of women did not have or receive an ITN. | Prevalence in cross-sectional survey [115]: *N. gonorrhoeae* = 9.7% *C. trachomatis* = 11.1% *T. vaginalis* = 21.3% 33.7% had at least one infection. |
| Pfizer [116] | Multicentre | All gravidae | 5.7% 68/1188 | 3.7% 45/1211 | - | - | - | - | 5.0% 57/1138 | 4.0% 47/1164 | Field workers verified installation of new ITNs during home visits following enrolment: AZCQ (98.5%) and SP (98.3%) | Per regulatory requirement, all participants for whom there was a missing birth outcome were categorised as a treatment failure (ITT analysis versus per protocol analysis) |

Note: (1) participants were given insecticide treated bed nets unless otherwise noted; (2) The control group in Papua New Guinea received SP+CQ per national policy

IPTp = Intermittent preventive treatment of malaria in pregnancy; SP = sulphadoxine-pyrimethamine; AZ = azithromycin; CQ = chloroquine; LBW = low birth weight; ITT = Intention to treat

## Epidemiology of STIs/RTIs in pregnancy

The infective agent of syphilis is *Treponema pallidum*, a highly motile, Gram-negative spirochete bacterium that can be transmitted from mother to child by placental invasion or through contact of a maternal syphilitic lesion during delivery. Although vertical transmission is uncommon prior to 18 weeks of gestation,[117] *T. pallidum* can gain access to the foetal compartment as early as 9 to 10 weeks.[123,124] In some populations, 40% of spontaneous abortions are attributable to untreated syphilis during pregnancy.[118,119] Syphilis infection is associated with an 18-fold increase in the risk of stillbirth (Risk ratio [RR] = 18.1; 95% CI: 5.5, 59.6), double the risk of intrauterine growth retardation (RR = 2.1; 95% CI: 1.0, 4.2), a six-fold increase in the risk of preterm birth (RR = 6.1; 95% CI: 2.5, 15.3), and treble the risk of LBW (RR = 3.3; 95% CI: 2.0, 5.4).[120]

Transmission of the Gram-negative diplococci bacterium *Neisseria gonorrhoeae* usually occurs from mother to child during delivery, although foetal infection can occur during prolonged rupture of the membranes. Maternal infection with *N. gonorrhoeae* was associated with a two-fold increase in the odds of preterm birth in the United States (OR = 2.0; 95% CI: 1.0, 4.0)[121] and a three-fold increase in Kenya (OR = 3.2; 95% CI: 1.3, 8.4).[122] These same studies also reported a statistically significant increase in LBW amongst babies born to infected women. A common co-infection with *N. gonorrhoeae, Chlamydia trachomatis* is also a Gram-negative bacterium and is associated with intrauterine growth retardation (OR = 2.4; 95% CI: 1.3, 4.2),[123] preterm delivery (OR 1.6; 95% CI: 1.0, 2.5),[123] LBW (OR = 2.7; 95% CI: 1.3, 5.7),[124] premature

rupture of the membranes (OR = 2.4; 95% CI: 1.1, 5.4),[124] and preterm birth (OR = 4.0; 95% CI: 1.7, 9.2).[124]

*Trichomonas vaginalis*, a protozoan parasite, is responsible for one-half of all curable STIs worldwide[125] and has been shown to increase the odds of preterm delivery between 30% (OR = 1.3; 95% CI: 1.1, 1.4),[126] 40% (OR = 1.4; 95% CI: 0.7, 2.8),[121] and 50% (OR = 1.5; 95% CI: 0.1, 8.1).[127] Infection by *T. vaginalis* increases the odds of LBW by 30% (OR = 1.3; 95% CI: 1.1, 1.5),[126] by 50% (OR = 1.5; 95% CI: 0.9, 2.6),[121] by two-fold (OR = 2.1; 95% CI: 1.0, 4.7).[128] Bacterial vaginosis is a syndrome caused by a disequilibrium in the vaginal microbiota with a concomitant decline in the number of naturally occurring lactobacilli, and is widely associated with adverse birth outcomes. Meta-analysis suggests that bacterial vaginosis increases the odds of a spontaneous abortion by ten-fold (OR = 9.9; 95% CI: 2.0, 49.3)[129] and has been shown to increase the risk of premature rupture of the membranes by 3.5-times (3.5 = RR; 95% CI: 1.4, 8.9).[130] One study in the United Kingdom associated bacterial vaginosis infection with a 13-fold increase in risk of preterm birth (RR = 13.1; 95% CI: 4.0, 42.6),[131] although most studies suggest that the risk of preterm birth attributable to bacterial vaginosis is increased by one- or two-fold, levels that are similar for LBW. The effects of these five curable STIs/RTIs on pregnancy outcomes are summarised in Table 4.

### Management of curable STIs/RTIs in pregnancy

As noted earlier, the WHO recommends syphilis screening and treatment for pregnant women at their first antenatal visit. For other curable STIs/RTIs, the WHO suggests using syndrome-based algorithms to guide diagnosis and treatment. The approach has

been useful amongst men for whom STIs/RTIs are more commonly symptomatic. In contrast, the vast majority of gonococcal (80%) and chlamydial (70% to 75%) infections amongst women are asymptomatic[125] and, therefore, never diagnosed. Consequently, the algorithm based on vaginal discharge syndrome has a low sensitivity (30% to 80%) and specificity (40% to 80%) for *N. gonorrhoeae* and *C. trachomatis* amongst pregnant women.[132-134] Syndromic management has slightly higher sensitivity for *T. vaginalis* (54% to 83%) and bacterial vaginosis (51% to 69%) with moderate specificity (40% to 54% for *T. vaginalis*; 40% to 58% for bacterial vaginosis).

## Table 4. Effect of curable STIs/RTIs on birth outcomes

| Reference | Country | Year(s) | Spontaneous abortion (95% CI) | Stillbirth (95% CI) | IUGR (95% CI) | PROM (95% CI) | Preterm birth (95% CI) | LBW (95% CI) |
|---|---|---|---|---|---|---|---|---|
| ***Treponema pallidum*** | | | | | | | | |
| Watson-Jones (2002) [120] | Tanzania | 1998-2000 | NR | 18 (5.5, 59.6) RR | 2.1 (1.0, 4.2) RR | NR | 6.1 (2.5, 15.3) RR | 3.3 (2.0, 5.4)[a] |
| Temmerman (1995 [135] | Kenya | 1991 | NR | 3.34 RR | NR | NR | NR | 4.01[a] |
| McDermott (1993) [136] | Malawi | 1987-1990 | NR | 10.98 | NR | NR | NR | NR |
| Donders (1993) [137] | South Africa | 1988 | NR | NR | NR | NR | 33%; 5 of 15 cases | NR |
| Elliott (1990) [122] | Kenya | 1985 | NR | NR | NR | NR | 1.4 (0.5, 4.1) | NR |
| Ratnam (1982) [119] | Zambia | NR | 42% of cases | NR | NR | NR | NR | NR |
| Williams (1923) [118] | United States | 1923 | 40% of cases | NR | NR | NR | NR | NR |
| ***Neisseria gonorrhoeae*** | | | | | | | | |
| Johnson (2011) [121] | United States | 1996-2002 | NR | NR | NR | NR | 2.0 (1.0, 4.0) | 0.8 (0.3, 2.3) |
| Donders (1993) [137] | South Africa | 1988 | NR | NR | NR | NR | 56%; 5 of 9 cases | *P*<0.005 |
| Elliott (1990) [122] | Kenya | 1985 | NR | NR | NR | NR | 3.2 (1.3, 8.4) | NR |
| ***Chlamydia trachomatis*** | | | | | | | | |
| Rours (2011) [138] | Nether-lands | 2003-2005 | NR | NR | NR | NR | 4.4 (1.3, 15.2)[a]; 2.7 (1.1, 6.5)[b]; 1.17 (0.6, 2.4)[c] | 1.0 (0.4, 2.2) |
| Silveira (2009) [139] | United States | 2005-2008 | NR | NR | NR | NR | 0.7 (0.4 to 1.4) | NR |
| Wilkowska-Trojniel (2009) [140] | Poland | 2003-2006 | 12 v 2% *P*=0.029 | NR | NR | NR | NR | NR |
| Blas (2007) [141] | United States | 2003 | NR | NR | NR | 1.5 (1.0, 2.2) RR | 1.5 (1.1, 2.0) RR | 1.1 (0.7, 1.7) |
| Odendaal (2006) [142] | South Africa | 2002-2003 | NR | NR | NR | NR | 22.2%; 8 of 36 cases vs. 10.4%; 32 of 307 cases; *P*=0.037 | NR |
| Johnson (2011) [121] | United States | 1996-2002 | NR | NR | NR | NR | 1.0 (0.6, 2.0) | 2.1 (1.0, 4.2) |
| Kovacs (1998) [143] | Hungary | 1994-1995 | NR | NR | 7.3 v 5.8% *P*>0.05 | 20 v 21% *P*>0.05 | NR | 15.5% vs. 13.2% *P*>0.05 |
| Donders (1993) [137] | South Africa | 1988 | NR | NR | NR | NR | 27%; 6 of 22 cases | NR |
| Elliott (1990) [122] | Kenya | 1985 | NR | NR | NR | NR | 0.7 (0.4, 1.4) | NR |
| Johns Hopkins (1989) [123] | United States | 1983-1985 | NR | NR | 2.4 (1.3 to 4.2) aOR | NR | 1.6 (1.0, 4.2) | NR |
| Gravett (1986) [124] | United States | 1983 | NR | NR | NR | 2.4 (1.7, 5.4) aOR | 4.0 (1.7, 9.2) aOR | 2.7 (1.3, 5.7) aOR |

## Table 4. Effect of curable STIs/RTIs on birth outcomes (continued)

| Reference | Country | Year(s) | Spontaneous abortion (95% CI) | Stillbirth (95% CI) | IUGR (95% CI) | PROM (95% CI) | Preterm birth (95% CI) | LBW (95% CI) |
|---|---|---|---|---|---|---|---|---|
| ***Trichomonas vaginalis*** | | | | | | | | |
| Johnson (2011) [121] | United States | 1996-2002 | NR | NR | NR | NR | 1.4 (0.7, 2.8) | 1.5 (0.9, 2.6) |
| Meis (1995) [127] | United States | 1992-1994 | NR | NR | NR | NR | 1.5 (0.1, 8.1) wk 24; 0.9 (0.2, 3.6) wk 28 | NR |
| Sutton (1999) [128] | DR Congo | 1989-1990 | NR | NR | NR | NR | NR | 2.1 (1.0, 4.7) |
| Minkoff (1984) [144] | United States | NR | NR | NR | NR | P<0.03 | NR | NR |
| Cotch (1997) [126] | United States | 1984-1989 | NR | NR | NR | NR | 1.3 (1.1, 1.4) | 1.3 (1.1, 1.5) |
| ***Bacterial vaginosis*** | | | | | | | | |
| Johnson (2011) [121] | United States | 1996-2002 | NR | NR | NR | NR | 1.3 (0.9, 2.1) | 1.1 (0.6, 1.8) |
| Svare (2006) [145] | Denmark | 1998-2002 | NR | NR | NR | NR | 2.5 (1.6, 3.9) | 2.0 (1.3, 2.9) |
| Watson-Jones (2007) [146] | Tanzania | 1997-2000 | NR | NR | NR | NR | 3.0 (1.3, 6.6) | NR |
| Leitich meta-analysis (2003) [129] | Multiple | Multiple | 9.9 (2.0, 49.3) | NR | NR | NR | 2.2 (1.5, 3.1) | NR |
| Meis (1995) [127] | United States | 1992-1994 | NR | NR | NR | NR | 1.4 (0.9, 2.05) wk 24; 1.8 (1.2, 3.0) wk 28 | NR |
| McGregor (1995) [130] | United States | 1991-1992 | NR | NR | NR | 3.5 (1.4, 8.9) RR | 1.9 (1.2, 3.0); 1.5 (0.7, 3.0)[e] RR | NR |
| Hillier (1995) [147] | United States | 1984-1989 | NR | NR | NR | 1.1 (0.8, 1.6) | 1.4 (1.1, 1.8) | 1.5 (1.2, 1.7) |
| Hay (1994) [131] | United Kingdom | NR | 5.5 (2.3, 13.3)[f] | NR | NR | NR | 13.1 (4.0, 42.6)[f] | NR |
| Elliott (1990) [122] | Kenya | 1985 | NR | NR | NR | NR | 1.0 (0.6, 1.8) | NR |
| Gravett (1986) [124] | United States | 1983 | NR | NR | NR | 2.0 (1.1, 3.7) | NR | 1.5 (0.8, 2.0) |

Results reported as odds ratios unless otherwise noted and 95% confidence intervals are in parentheses; IUGR=Intrauterine growth retardation; PROM=Premature rupture of membranes; NR=Not reported; RR=Risk ratio; [a] Preterm delivery before 32 weeks; [b] Preterm delivery before 35 week; [c] Preterm delivery before 37 weeks; [d] Bacterial vaginosis at 16-20 weeks; [e] Bacterial vaginosis at 28-32 weeks; [f]Intermediate flora (Nugent score 4-7) and bacterial vaginosis (Nugent score 7-10); aOR = adjusted odds ratio

## Rationale and research questions

The rationale underlying the five research questions to be addressed in this doctoral thesis is presented below along with a description of current knowledge gaps.

### Q 1. Malaria transmission intensity threshold

The antenatal intervention of IPTp-SP was formally recommended by the WHO in 2004 to protect against the adverse effects of malaria infection amongst pregnant women who reside in areas of moderate (stable) to high transmission.[20] Meta-analysis suggests that chemoprophylaxis reduces the incidence of LBW amongst primi- and secundigravidae by 27% (RR = 0.73; 95% CI: 0.61, 0.87; 8 trials; N = 3,619).[21] The same review reported a trend of protection against LBW in favour of IPTp-SP use amongst multigravidae, but neither the three trials included, nor the pooled analysis demonstrated standard levels of statistical significance (RR = 0.86, 95% CI 0.64 to 1.17; 3 trials; N = 2,743 participants). However, IPTp-SP does not feature in the WHO malaria elimination guidelines for countries with moderate and low endemicity.[148] Thus, what is the malaria transmission intensity-threshold below which IPTp-SP may no longer protect against the incidence of LBW and the intervention could be withdrawn?

### Q 2. A581G prevalence threshold

The evidence supporting the original IPTp-SP policy recommendation in 2004 by the WHO originated from clinical trials and field experience in Kenya[74,75] and Malawi[38,76] dating to the 1990s when parasite epigenomics was in its infancy.

Accumulated mutations in the *P. falciparum* genes that encode *Pfdhfr* and *Pfdhps*, alleles implicated in SP resistance[25,149-151] were present during this same period. Mutations at seven *Pfdhfr* codons (16, 50, 51, 59, 108, 140 and 164) and five *Pfdhps* codons (436, 437, 540, 581 and 613) have since been isolated as molecular markers for SP resistance.[152] The protection conferred by IPTp-SP against LBW has been shown to decrease as the population prevalence of the *Pfdhps* K540E mutation increases—a proxy for the resistant quintuple *Pfdhfr* and *Pfdhps* mutants.[153] Of particular concern, however, in areas where the *Pfdhps* A581G mutation has emerged alongside the *Pfdhfr* and *Pfdhps* quintuple mutant, producing sextuple mutant parasites, IPTp-SP fails to inhibit the growth of parasites.[91] Within the context of declining parasite sensitivity to SP, is there a threshold of *P. falciparum* resistance to SP defined by the prevalence of *Pfdhps* mutation at codon A581G above which IPTp-SP may no longer protect against the incidence of LBW?

## Q 3. Effect of IPTp-SP on malaria and curable STIs/RTIs in pregnancy

Desai and colleagues recently reported outcomes from the first investigation of an artemisinin combination treatment, dihydroartemisinin–piperaquine (DP), in IPTp.[154] Women received IPTp-SP or IPTp-DP, or were screened for peripheral parasitaemia and treated, if infected, with DP. The trial was conducted in a region of western Kenya where 96% of parasites had the quintuple mutation and 5.8% carried the A581G mutation. This trial was designed to test parasite clearance with a sample size capable of detecting a 50% decrease in maternal malaria infection at delivery, defined as a composite of peripheral or placental parasitaemia as detected by placental histology, microscopy, or RDT. The prevalence of malaria infection at delivery was lowest

amongst women given IPTp-DP, 3.0% (15 of 457), followed by recipients of IPTp-SP, 10.2% (47 of 459), compared to 12.6% (57 of 452) of women who were provided screening and treatment services.

The incidence of any adverse birth outcome – a composite of small for gestational age, LBW, preterm birth or foetal loss – was comparable between recipients of IPTp-DP and IPTp-SP, 10.6% (48 of 452) and 11.9% (54 of 454), respectively. In contrast, stillbirth was experienced by 0.9% (4 of 452) of women in the IPTp-DP group compared to 3.5% (16 of 453) recipients of IPTp-SP, a statistically significant observation ($P$ = 0.013). IPTp-DP reduced the incidence of clinical malaria during pregnancy by 83.9% (37.9 vs 6.1 episodes) and the risk of maternal anaemia by 22.0% at delivery (crude prevalence ratio 0.78, 95% CI 0.64 to 0.96) compared to IPTp-SP. Secondary analysis showed that women given intermittent screening and treatment had the highest prevalence of peripheral or placental malaria at delivery: 42.5% (199 of 452) women versus 30.6% (140 of 457) women in the IPTp-DP group and 36.2% (166 of 459) women in the IPTp-SP group. Interestingly, despite the protective effect conferred by IPTp-DP against many secondary endpoints, recipients of IPTp-SP delivered babies weighing on average (corrected) 87.3 grams more, a statistically significant observation ($P$ = 0.001). This was reflected in superior birth weight for gestational age ($Z$-score) among mothers who received IPTp-SP versus IPTp-DP ($P$ = 0.001). The incidence of LBW, small-for-gestational age, and preterm birth were slightly lower amongst women given IPTp-SP compared to IPTp-DP, but the differences were too small to draw conclusions in these three secondary endpoints.

Sulphadoxine-pyrimethamine has been used to prevent *Pneumocystis jiroveci* pneumonia and *Toxoplasma gondii* infection.[155] Sulphadoxine is pharmacologically related to sulphamethoxazole, a compound co-formulated with trimethoprim for the treatment of urinary tract infections and to prevent *P. jiroveci* amongst HIV-infected patients.[156] Prior to the development of penicillin, sulphonamides were used to treat *N. gonorrhoeae.*[157] Based on historical use, sulphonamides could exert some effect on *C. trachomatis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, amongst many other pathogens.[158] Sulphonamides have also been used to treat *Gardnerella vaginalis*,[159] a bacterium that is commonly found in high concentrations amongst women with bacterial vaginosis.

Thus, because IPTp-SP was more protective against the incidence of LBW, small-for-gestational age, and preterm birth, might the protection conferred to pregnant women by IPTp-SP be explained, in part, by an effect against curable STIs/RTIs as well as malaria infection? If so, what is the burden of STIs/RTIs in areas where IPTp-SP is being implemented?

## Q 4. Prevalence of malaria and curable STIs/RTIs in pregnancy

The dual burden of malaria and curable STIs/RTIs in pregnancy has never before been characterised by systematic review and meta-analysis. In the case of malaria infection during pregnancy, two seminal reviews have defined the burden in the sub-Saharan Africa. Brabin's 1983 paper summarised prevalence estimates of peripheral and placental parasitaemia from 12 sub-Saharan studies conducted between 1925 and 1978.[160] Peripheral parasitaemia ranged from 5.5% (N = 3,582)[161] in Freetown, Sierra Leone to 76.0% (N = 50)[162] in the former Belgian Congo (present-day Kisangani,

Democratic Republic of Congo). The low and high estimates of placental parasitaemia were also from the former Belgian Congo, ranging between 2.0% (N = 55)[163] in Leopoldville (present-day Kinshasa) and 74.0% (N = 50) from Stanleyville.[162]

The 2001 review by Steketee *et al.* contained data from 20 sub-Saharan countries collected between 1985 and 2000.[18] The lowest maternal parasite prevalence across 16 studies from East and Southern Africa was 10.2% (N = 503) from Kilifi, Kenya.[30] However, authors of the Kilifi study stated their estimate was based on placental blood smears, and went on to report that 77.4% (n = 53) of women had placental infections which represents the highest point estimate included in the review. The lowest prevalence measure from East and Southern Africa was in Chikawa, Malawi, where 19.0% (N = 3,913) of pregnant women had peripheral malaria infections.[164] In West and Central Africa, the studies that had the highest prevalence of placental parasitaemia did not also report peripheral measures of infection. Estimates ranged from 19.0% (N = 904) in Banfora, Burkina Faso[165] to 57.8% (N = 120) in Ebolowa, Cameroon.[166]

The review by Brabin separated peripheral versus placental infection estimates, while Steketee *et al.* did not. Neither review contained data more recent than the year 2000, nor attempted to pool the prevalence data. Moreover, both were published before the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) were created to standardise methods of conducting systematic reviews.[167] Thus, a contemporary systematic review and meta-analysis of malaria infection in pregnancy is overdue. Moreover, pooled estimates stratified by East and

West African sub-regions can assist policymakers and programme managers with planning and advocacy efforts related to malaria infection during pregnancy.

As for the burden of curable STIs/RTIs, there did not appear to have been a prior systematic review and meta-analysis of data from pregnant women attending ANC facilities in sub-Saharan Africa at the time this investigation was conducted. A review by Mullick *et al.*[168] in 2005 does contain a summary of point estimates for curable STIs/RTIs amongst pregnant women, although no attempt was made to pool the data. Thus, a systematic review to quantify the dual burden of malaria infection and STIs/RTIs in sub-Saharan Africa is needed to make rational choice of drug to replace SP in areas where the drug is failing to protect against adverse birth outcomes.

## Q 5. Effect of azithromycin on curable STIs/RTIs in pregnancy

Potential replacements for SP will likely need to confer protection against adverse birth outcomes attributable to malaria infection and other non-malarial causes. Azithromycin is a broad-spectrum antibiotic and a leading partner-drug for use with DP in IPTp. Azithromycin is the WHO-recommended treatment for pregnant women with *C. trachomatis* infections and *in vivo* studies amongst high- and low-risk populations with syphilis and *N. gonorrhoeae* suggest potential therapeutic value, despite shifts during the past two decades of *in vitro* sensitivity testing. It is relatively unknown whether azithromycin might confer some protective effect against *T. vaginalis* or bacterial vaginosis. In the context of a high dual burden of malaria infection and curable STIs/RTIs amongst pregnant women, would azithromycin be an efficacious drug to include as part of IPTp?

## Methods outline and monograph structure

The methods used to address each research question are described in detail within their respective chapters. A brief outline is presented herein. Questions related to estimating the lower threshold of malaria transmission intensity and the upper prevalence threshold of the SP resistance were addressed first by a systematic review of published literature followed by relevant pooled analyses. The question about the effect of IPTp-SP on adverse birth outcomes attributable to malaria infection and curable STIs/RTIs was determined by secondary analysis of data collected for a prospective cohort study of the burden of malaria and curable STIs/RTIs in Zambia. The question related to the prevalence of malaria and curable STIs/RTIs in sub-Saharan Africa was addressed by a systematic review and meta-analyses, as was the last question concerning the efficacy of azithromycin against curable STIs/RTIs.

The next section of the monograph is entitled, "Methods, results and discussion by research question" and contains five sub-sections, one for each of the five research questions. Within each of the five sub-sections, the methods, results and discussion are presented in their entirety. The section thereafter, "Monograph discussion", contains a broader discourse that relates to the research questions as a whole. The last section, "Monograph summary and conclusions", highlights key findings from these research questions and closes with a research pathway forward.

## Methods, results and discussion by research question

### Q 1. Malaria transmission intensity threshold

#### Methods

Estimating the malaria transmission intensity below which IPTp-SP no longer protects against LBW would be straight forward if there were data from multiple placebo-controlled randomised clinical trials (RCTs) that had been conducted in malaria-endemic areas characterised by narrowly different strata of transmission. However, it would only be ethical to conduct such trials in countries that have not already adopted IPTp-SP as policy. Thus, the question must be addressed using data extracted from trials as found in the literature alongside robust estiamtes of transmission intensity at the trial sites.

For this, data from IPTp-SP studies were first compiled by systematic review of published literature from sources that included PubMed, MEDLINE, EMBASE, the WHO International Clinical Trials Registry, and reference lists from the identified texts. The medical subject headings and free text terms used are shown in Table 1.1 below.

**Table 1.1 Medical subject headings and free text terms**

| Medical subject headings | Free text terms |
|---|---|
| Topic=("Malaria") AND Topic=(Prenatal OR Antenatal OR Pregnancy OR Pregnant) OR Topic=(women) AND Topic=(Africa) | Malaria AND Pregnant AND Africa<br>Malaria AND Pregnancy AND Africa<br>Screen AND Malaria AND Pregnant AND Africa<br>Screen AND Malaria AND Pregnancy AND Africa<br>Test AND Malaria AND Pregnant AND Africa<br>Test AND Malaria AND Pregnancy AND Africa |

Studies that had reported the: (i) proportion of pregnant women who were exposed to $\geq$ 2 doses of IPTp-SP compared to placebo, or to no doses of IPTp-SP, and (ii) the

incidence of LBW by treatment group and gravidae were included in this analysis. EndNote X7 software was used to important and manage records. Duplicates were removed and the remaining records were screened against eligibility criteria. Full-text articles were then reviewed against the same criteria. Data were abstracted without blinding author names or publication titles, and then appraised for quality using methods developed by Grading of Evidence, Assessment, Development and Evaluation (GRADE) Working Group. The resulting data set was analysed using Stata/IC version 13 software. This involved pooling of LBW estimates by gravidae (primi- and secundigravidae versus multigravidae) and exposure to IPTp-SP doses ( $\geq$ 2 doses versus placebo or no doses).

The optimal time to measure transmission intensity in IPTp-SP trials would be at enrolment when maternal parasitaemia is likely highest. Evidence suggests that parasite densities peak between gestational weeks nine and 16, tapering until delivery.[169] This is because the prevalence of malaria infection estimated from samples colleced at ANC booking, rather than later in pregnancy, would not be biased by cumulative IPTp-SP exposure during the antenatal period, particularly near-to-term dosing that would skew downward the meausrements of infection. Despite these reasons, parasitaemia is rarely measured at enrolment. Active screening with miscroscopy or rapid diagnostic tests obliges investigators to treat women who are found to have asymptomatic parasitaemia with an artemisinin combination treatment, thereby fundamentally altering the investigation. Thus, if parasitaemia is measured, it is done almost exclusively at delivery and, for reasons stated, fails to reflect the true

transmission intensity. Thus, a proxy measure is needed to define malaria transmission intensity at the trial sites to answer this research question.

To identify a proxy measure of the prevalence of malaria infection at the IPTp-SP study sites, data were used from the Malaria Atlas Project (MAP),[170] the largest repository of malaria prevalence estimates in the world. These data were used in two ways to estimate the malaria transmission intensity that was likely to have been present in each IPTp-SP study site. The first approach was simply to apply the 2007 prevalence estimates of *P. falciparum* infection amongst children 2 to 10 years of age (2007 *Pf*$PR_{2-10}$) as calculated by the MAP for the same locations where IPTp-SP studies had been conducted. However, all IPTp-SP studies identified by the systematic review had been conducted prior to 2007, with the exception of one multi-year trial that ended in 2007. Thus, a second method was used to identify data that aligned better with the years of the IPTp-SP trials.

National cross-sectional survey datasets were obtained from MAP of *P. falciparum* prevalence estimates from each of the 12 countries where the IPTp-SP trials had been conducted, and neighbouring countries if pregnancy trials had been conducted near border areas. Four matching rules based on space and time parameters shown in Table 1.2 were then used to select point estimates from these surveys that were most likely to reflect the malaria transmission intensity to which pregnant women had been exposed. However, before applying these matching rules, geospatial coordinates were obtained for each IPTp-SP study and cross-sectional survey using the GEOnet Names Server.[171] ArcGIS 10 software was then used to calculate straight-line distances

between the specific locations where IPTp-SP studies and cross-sectional surveys had been conducted. Doing so allowed matching the point estimates from surveys to within < 100 miles of IPTp-SP studies as described in rule 1. This same criterion of < 100 miles had been used in previous malaria modelling of pregnancy and paediatric data.[172] In the same way, elevation estimates were obtained from the Consortium for Spatial Information[173] for locations of the IPTp-SP studies and survey data. These estimates were used as part of an elevation criterion in rule 3 based on evidence that malaria infection in children declines sharply at altitudes $\geq$ 1,200 metres above sea level compared to lower elevations.[174] A temporal criterion was included, as well, in the matching rules stipulated survey data to have been collected within plus or minus two years of the IPTp-SP trials. To account for the possibility that IPTp-SP studies and cross-sectional surveys may have been conducted in proximate locations that happened to have had very different intensities of malaria transmission, the matching rules required that cross-sectional surveys and IPTp-SP trials had to have been conducted in areas from the same malaria transmission category as designated by MAP: high ( > 40% *P. falciparum* prevalence amongst 2 to 10 year olds), intermediate (5 to 40%), and low ( < 5%).

**Table 1.2 Matching rules**

| Rules | Criteria |
|---|---|
| 1 | **Prevalence estimates will be paired to IPTp-SP studies if they were both conducted:** |
| | a. In an area with the same risk of malaria infection (high, intermediate or low), AND |
| | b. Within two years (±) of each other, AND |
| | c. < 100 miles of each other. |
| 2 | **Prevalence estimates will be paired to IPTp-SP studies if they were both conducted:** |
| | a. In an area with the same risk of malaria infection (high, intermediate or low), AND |
| | b. Within two years (±) of the pregnancy study, AND |
| | c. In the same country. |
| 3 | **Prevalence estimates will be paired to IPTp-SP studies if they were both conducted:** |
| | a. In an area with the same risk of malaria infection (high, intermediate or low), AND |
| | b. At the same elevation, either $\leq$ 1200 metres OR > 1201 metres, AND |
| | c. In the same sub-region of Africa (East/Southern OR West/Central) |
| 4 | **If no prevalence estimates can be paired, then IPTp-SP studies will be matched to the 2007 *Pf*PR$_{2-10}$ estimate** |

To reflect the gravidae-specific nature of parasitaemia amongst pregnant women when pairing prevalence estimates of malaria infection, assumptions were made based on old reports from Sierra Leone[161] and Kenya[160] that suggest levels of parasitaemia amongst children are comparable to maternal parasitaemia as presented in Table 1.3.

**Table 1.3 Age structure of paediatric data matched to gravidity**

| Paediatric descrition | Age structure | Gravidity |
|---|---|---|
| Infancy | Birth to < 1 | Primigravidae |
| Childhood | 1 to 4 | Segundigravidae |
| School-aged | 5 to 15 | Multigravidae |

Matching rules were then applied sequentially. If survey data could not be matched to an IPTp-SP study using rule 1, then rule 2 was applied, and so on until all point prevalence estimates had been identified from the survey data that could be considered the best possible matches for each IPTp-SP study. Survey data paired to IPTp-SP studies under rules 1, 2 or 3 were pooled using random-effects models.[175] If survey data could not be matched under these rules, then the 2007 *Pf*PR$_{2-10}$ estimate

for the same location where the IPTp-SP study had been conducted was imposed on the model under rule 4. Whether based on the 2007 MAP estimate, or the pooled prevalence estimate, standard meta-regression models were applied to LBW outcomes from the IPTp-SP studies.

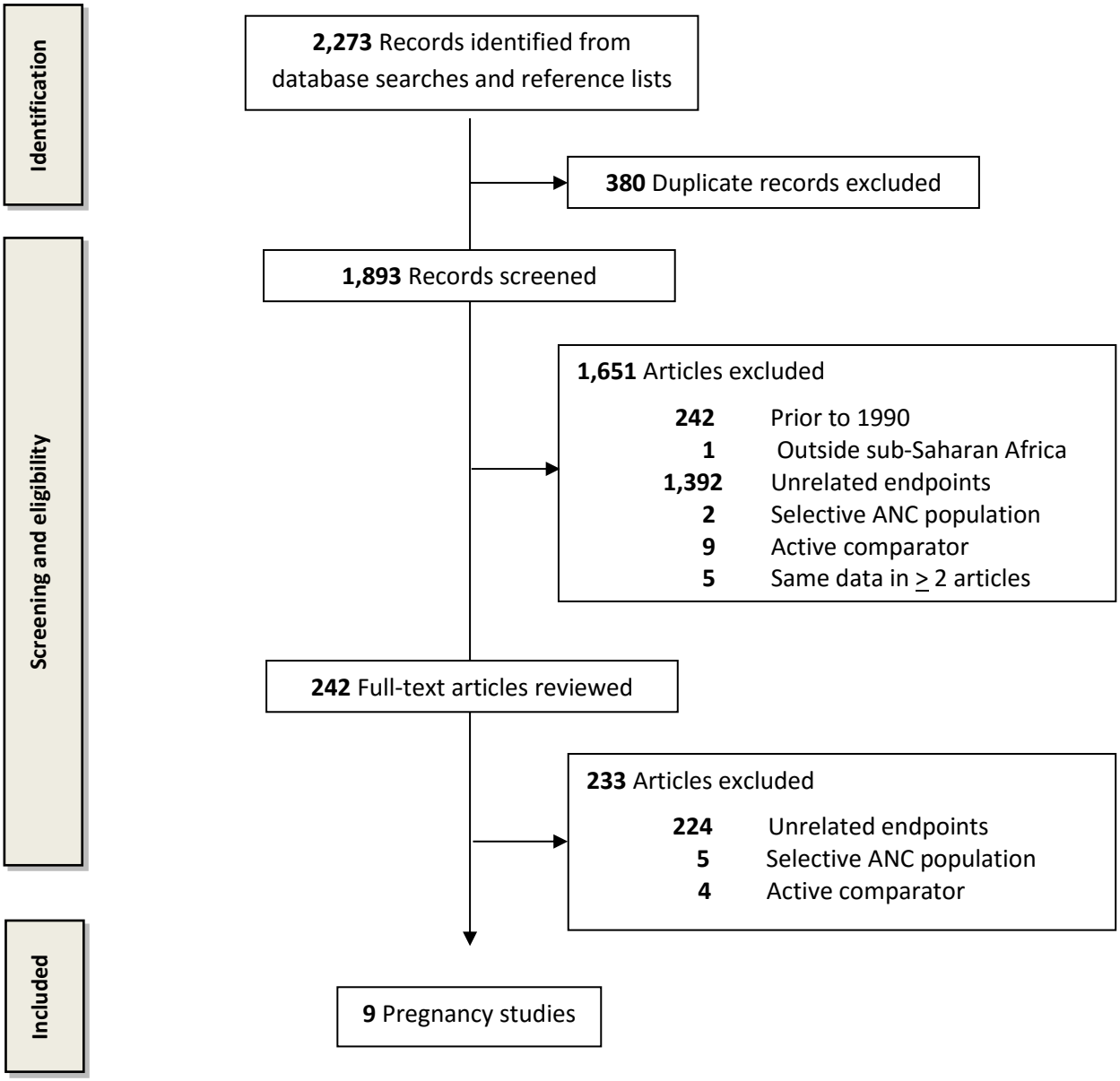
## Results

### Systematic review

A total of nine pregnacy studies met inclusion criteria involving 10,279 pregnant women. Figure 1.1, the PRISMA flowchart for the systematic reivew, summarises the selection process, the number of studies excluded and the reasons for their exclusion according to PRISMA guidelines. Pregnancy studies stratified by gravidae that met inclusion criteria are summarized in Table 1.4.[77,80,81,176-181] As shown in Figure 1.2, the PRISMA flowchart for matching studies, data on LBW came from seven studies and 12 unique sites for primi- and secundigravidae who were exposed to $\geq$ 2 doses of IPTp-SP (n = 2,314) versus placebo or no doses (n = 1,954). In contrast, seven studies from 10 unique sites reported LBW outcomes amongst multigravidae who received $\geq$ 2 doses of IPTp-SP (n = 2,941) compared to placebo or no doses (n = 3,070).

Funnel-plot analysis in Figure 1.3 suggests that IPTp-SP studies that were comparatively small and failed to protect against LBW may have been under-represented in the sample. Risk of bias among individual studies was appraised using GRADE methods and is summarised in Table 1.5. Figure 1.4 illustrates the risk of bias within these individual IPTp-SP studies, and Figure 1.5 shows the combined risk of bias across IPTp-SP studies, both of which were prepared using RevMan 5.2 software.

**Figure 1.1 PRISMA flowchart for systematic review**



## Table 1.4 Summary of included studies by gravidae

| References | Countries | Sites | Years | Type | IPTp-SP (2 doses or more) | | | | Placebo or no IPTp-SP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | No. LBW | No. Weighed | LBW % | 95% CI | No. LBW | No. Weighed | LBW % | 95% CI |
| **Primi- and secundigravidae** | | | | | | | | | | | | |
| Gies (2009) [177] | Burkina Faso | Boromo | 2004-06 | 1 | 104 | 812 | 12.8 | 10.5, 15.1 | 19 | 52 | 36.5 | 23.5, 49.6 |
| Likwela (2012) [176] | DR Congo | Kisangani | 2007 | 2 | 2 | 28 | 7.1 | -2.4, 16.7 | 7 | 12 | 58.3 | 30.4, 86.2 |
| Likwela (2012) [176] | DR Congo | Mikalayi | 2007 | 2 | 1 | 28 | 3.6 | -3.3, 10.5 | 11 | 56 | 19.6 | 9.2, 30.0 |
| Likwela (2012) [176] | DR Congo | Rutshuru | 2007 | 2 | 11 | 94 | 11.7 | 6.0, 20.0 | 5 | 15 | 33.3 | 9.5, 57.2 |
| Menendez (2008) [77] | Mozambique | Manhiça | 2003-05 | 1 | 29 | 133 | 21.8 | 14.8, 28.8 | 25 | 121 | 20.7 | 13.5, 27.9 |
| Ndyomugyenyi (2011) [81] | Uganda | Kabale | 2004-07 | 1 | 16 | 313 [a] | 5.1 | 2.7, 7.6 | 18 | 329 | 5.5 | 3.0, 7.9 |
| Ramharter (2007) [178] | Gabon | Lambaréné | 2006 | 2 | 4 | 49 | 8.2 | 0.5, 15.8 | 6 | 30 | 20.0 | 5.7, 34.3 |
| Ramharter (2007) [178] | Gabon | Libreville | 2006 | 2 | 14 | 168 | 8.3 | 4.2, 12.5 | 19 | 106 | 17.9 | 10.6, 25.2 |
| Ndyomugyenyi (2011) [81] | Uganda | Kabale | 2004-07 | 1 | 22 | 287 [b] | 7.7 | 4.6, 10.7 | 16 | 277 | 5.8 | 3.0, 8.5 |
| Sirima (2006) [180] | Burkina Faso | Koupéla | 2004 | 2 | 21 | 173 [a] | 12.1 | 7.3, 17.0 | 22 | 183 | 12.0 | 7.3, 16.7 |
| Sirima (2006) [180] | Burkina Faso | Koupéla | 2004 | 2 | 6 | 46 [c] | 13.0 | 3.3, 22.8 | 23 | 186 | 12.4 | 7.6, 17.1 |
| van Eijk (2004) [179] | Kenya | Kisumu | 1999-2000 | 1 | 10 | 122 | 8.2 | 3.3, 13.1 | 78 | 513 | 15.2 | 12.1, 18.3 |
| **Sub-totals** | | | | | 241 | 2,253 | | | 249 | 1,880 | | |
| **Multigravidae** | | | | | | | | | | | | |
| Likwela (2012) [176] | DR Congo | Kisangani | 2007 | 2 | 4 | 59 | 6.8 | 0.4, 13.2 | 9 | 37 | 24.3 | 10.5, 38.1 |
| Likwela (2012) [176] | DR Congo | Mikalayi | 2007 | 2 | 1 | 86 | 1.2 | -1.1, 3.4 | 24 | 307 | 7.8 | 4.8, 10.8 |
| Likwela (2012) [176] | DR Congo | Rutshuru | 2007 | 2 | 28 | 397 | 7.1 | 4.5, 9.6 | 11 | 162 | 6.8 | 2.9, 10.7 |
| Mbaye (2006) [80] | The Gambia | Farafenni | 2003-04 | 1 | 51 | 931 | 5.5 | 4.0, 6.9 | 63 | 917 | 6.9 | 5.2, 8.5 |
| Menendez (2008) [77] | Mozambique | Manhiça | 2003-05 | 1 | 29 | 361 [e] | 8.0 | 5.2, 10.8 | 34 | 375 | 9.1 | 6.2, 12.0 |
| Ndyomugyenyi (2011) [81] | Uganda | Kabale | 2004-07 | 1 | 40 | 610 [b] | 6.6 | 4.6, 8.5 | 37 | 631 | 5.9 | 4.0, 7.7 |
| Ndyomugyenyi (2011) [81] | Uganda | Kabale | 2004-07 | 1 | 21 | 347 [d] | 6.1 | 3.5, 8.6 | 27 | 325 | 8.3 | 5.3, 11.3 |
| Rogerson (2000) [181] | Malawi | Blantyre | 1997-99 | 2 | 30 | 291 | 10.3 | 6.8, 13.8 | 50 | 218 | 22.9 | 17.4, 28.5 |
| Sirima (2006) [180] | Burkina Faso | Koupéla | 2004 | 2 | 2 | 17 | 11.8 | -3.6, 27.1 | 7 | 61 | 11.5 | 3.5, 19.5 |
| van Eijk (2004) [179] | Kenya | Kisumu | 1999-2000 | 1 | 2 | 36 | 5.6 | -1.9, 13.0 | 23 | 232 | 9.9 | 6.1, 13.8 |
| **Sub-totals** | | | | | 208 | 3,135 | | | 285 | 3,265 | | |

Note: Presented in alphabetical order based on reference; IPTp-SP = Intermittent preventive treatment of malaria in pregnancy using sulphadoxine-pyrimethamine; LBW = Low birth weight; CI = Confidence interval; NA = Not applicable; Study type 1 = Randomised trial and type 2 = Observational study; [a] = primigravidae; [b] = secundigravidae; [c] = 2-4 pregnancies; [d] = 5 or more pregnancies; [e] = 1-3 pregnancies (likely to have contained some secundigravidae) and 4 or more pregnancies

**Figure 1.2 PRISMA flowchart for matching studies**

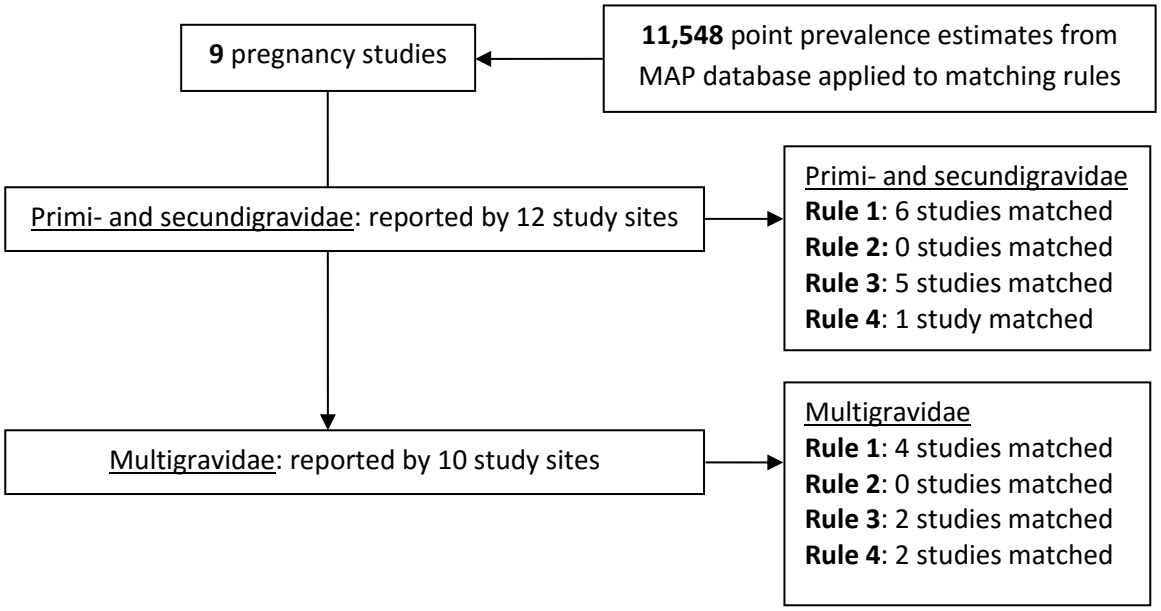
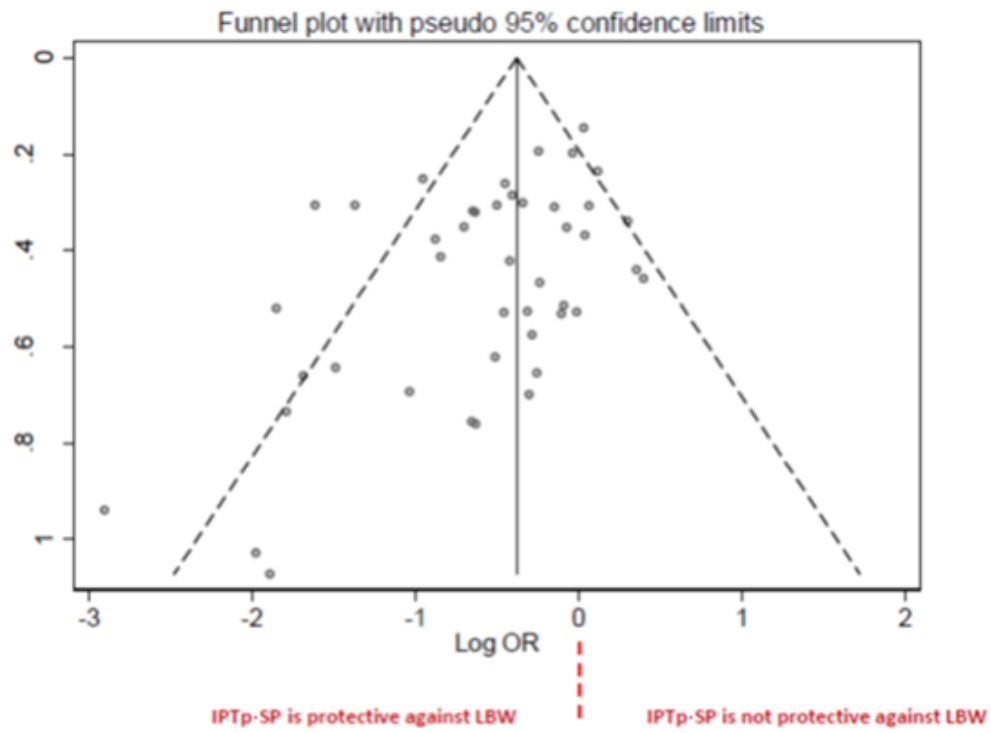


**Figure 1.3 Funnel plot**




This figure is a funnel plot of low birth weight estimates from pregnancy studies identified through systematic review and that stratified results by gravidae. Data points extracted from the pregnancy studies show that SP-IPTp is protective against LBW and, therefore, the midline of the funnel has shifted to the left of the number zero. Larger studies cluster around the upper area of the funnel as expected, reflecting smaller standard errors in these studies and within the 95% CIs. In contrast, small studies that reported the failure of IPTp-SP to protect against the incidence of LBW may have been under-represented in the sample of eligible pregnancy studies. This is reflected by the comparatively empty space towards the bottom right-hand side of the funnel.

**Table 1.5 Risk of bias amongst individual pregnancy studies**

| Reference | Type of bias | Description | Risk | Support for judgement of bias |
|---|---|---|---|---|
| Gies (2009) [177] | Selection | Random sequence generation | High | Although not randomised at the individual level, the study involved 12 peripheral health centres that had been selected for a community-based trial on IPTp-SP effectiveness; four health centres implemented IPTp-SP, while the others did not and served as controls. There was a statistically significant difference reported ($P < 0.05$) in the proportion of pregnant women residing > 5 km from the nearest health facility and the number of SP doses given: 0 doses (76.5%; 62/81) and $\geq$ 2 doses (23.0%; 202/877). |
| | Selection | Allocation concealment | Unclear | All primigravidae and secundigravidae enrolled in the study were identified by field assistants through monthly rounds in the villages. It is unclear whether the absence of allocation concealment introduced bias. |
| | Performance | Blinding of participants and personnel | Unclear | It is not clear how participants and personnel were blinded in this community-based trial. |
| | Detection | Blinding of outcome assessment | Low | Although outcome assessment did not appear to be blinded, newborns were weighed to the nearest 25 g as soon as possible after birth using a hanging scale; weights obtained 1–8 days after delivery were corrected for physiological change. |
| | Attrition | Incomplete outcome data | Low | The proportion of women lost to follow-up was low at 8.4% (158/1,883). Women not included into the analysis (9.6%; 181/1,883) did not differ in parity and age from women included. |
| | Reporting | Selective reporting | Low | There did not appear to be evidence of selective reporting. |
| | Other | | Low | No other sources of potential bias were identified. |

**Table 1.5 Risk of bias amongst individual pregnancy studies (continued)**

| Reference | Type of bias | Description | Risk | Support for judgement of bias |
|---|---|---|---|---|
| Likwela (2012) [176] | Selection | Random sequence generation | Unclear | All women who gave birth in 2007 in the study maternity clinics, attended ANC at least once during pregnancy, and whose antenatal card was available for analysis were included in this study. Demographic and anthropometric characteristics of women are not stratified according to IPTp-SP exposure and, therefore, it is unclear whether there was selection bias. |
| | Selection | Allocation concealment | Low | Authors noted that each maternity unit kept the ANC cards for all pregnant women from their catchment area. Hospital records and ANC cards were fully reviewed and the information transcribed. Although this does not conceal group allocation, bias was not necessarily introduced as a result of these procedures. |
| | Performance | Blinding of participants and personnel | Low | Newborns were weighed by a standard procedure in the three maternity wards using mechanical baby scales with a precision of 10 g. As reported, “The tendency of rounding the birth weight probably occurred in all three sites and should not significantly affect the results." |
| | Detection | Blinding of outcome assessment | Low | Analysis appeared to minimise the potential for detection bias. |
| | Attrition | Incomplete outcome data | Unclear | As a retrospective analysis of birth weight following exposure to IPTp-SP, it is unclear how data may be incomplete and contribute to attrition bias. |
| | Reporting | Selective reporting | Low | There is no evidence of selective reporting and a dose-response relationship was observed across gravidae. |
| | Other | | Unclear | It is unclear how a statistically significant difference in the age of women between sites in Kisangani and Mikalayi may affect pooled outcomes. |

**Table 1.5 Risk of bias amongst individual pregnancy studies (continued)**

| Reference | Type of bias | Description | Risk | Support for judgement of bias |
|---|---|---|---|---|
| Mbaye (2006) [80] | Selection | Random sequence generation | Low | Random sequence was maintained with the use of group assignments in locks of 12. |
| | Selection | Allocation concealment | Low | As a placebo-controlled trial, SP and placebo tablets were identical in shape and colour, and were pre-packed in envelopes that had been placed in a wallet bearing the subject's number and packet number. |
| | Performance | Blinding of participants and personnel | Low | Identical SP and placebo tablets were used. |
| | Detection | Blinding of outcome assessment | Unclear | Outcome assessment was blinded, although the method of measuring birth weight was not mentioned. It is unclear whether the fact that 87% of newborns were weighed between 3 and 5 days after birth introduced any detection bias. |
| | Attrition | Incomplete outcome data | Low | Attrition rate was not unlike other IPTp-SP trials. Loss to follow up of 17.1% (459/2,688) overall, 16.6% (223/1,346) in the SP group and 17.6% (236/1,342) in the placebo group. |
| | Reporting | Selective reporting | Low | There is no apparent risk of selective reporting. |
| | Other | | Unclear | It is unclear what bias, if any, could have been introduced given the difference in ages amongst women 40-50 in the SP vs. placebo groups, 61% (28/1,346) vs. 39% (18/1,342), respectively. |

**Table 1.5 Risk of bias amongst individual pregnancy studies (continued)**

| Reference | Type of bias | Description | Risk | Support for judgement of bias |
|---|---|---|---|---|
| Menendez (2008) [77] | Selection | Random sequence generation | Low | A computer-generated sequential list contained the study numbers linked to treatment identification letters that were randomly ordered in blocks of 10. |
| | Selection | Allocation concealment | Low | SP and placebo tablets were 'identical in shape and colour' |
| | Performance | Blinding of participants and personnel | Low | Blinding was maintained with the use of placebo tablets. |
| | Detection | Blinding of outcome assessment | Low | Although outcome assessment was not blinded, group allocation was maintained and newborns were weighed on a digital scale, accurate to the nearest gram. |
| | Attrition | Incomplete outcome data | Low | Loss to follow up was minimal. In the SP group 6.8% (35/515) of women did not receive 2 doses and birth weight was not measured for 1.4% (7/501) live births, whereas 5.6% (29/515) of women in the placebo group did not receive 2 doses and birth weight was not measured for 1.4% (7/503) live births. |
| | Reporting | Selective reporting | Low | No evidence of selective reporting and the study protocol is available. |
| | Other | | Unclear | A statistically significant difference in the prevalence of syphilis was reported between the IPTp-SP and placebo groups, 14% (72/515) vs. 10% (50/515). There was no discussion as to whether women were informed of the syphilis status, offered a referral or treatment and, if treatment, what therapy was administered. |

**Table 1.5 Risk of bias amongst individual pregnancy studies (continued)**

| Reference | Type of bias | Description | Risk | Support for judgement of bias |
|---|---|---|---|---|
| Ndyomugyenyi (2011) [81] | Selection | Random sequence generation | Low | Selection bias was minimised by using a computer-generated random number list with individually sealed envelopes, arranged in blocks of 12. |
| | Selection | Allocation concealment | Low | Tablets of SP or placebo, identical in shape and colour, were used. |
| | Performance | Blinding of participants and personnel | Low | All study participants, health staff and researchers were blind to drug assignment (SP or placebo), and newborns were weighed on a digital scale that was accurate to the nearest 10 g. |
| | Detection | Blinding of outcome assessment | Low | Data were examined to identify any imbalances in covariates and potential confounders in a blinded manner. |
| | Attrition | Incomplete outcome data | Low | 5,775 women were assigned to treatment groups; 4,713 women contributed to the final analysis of the primary endpoint. Attrition was 18.4% overall. However, data on birth weight were recorded for > 90% of women enrolled into the study |
| | Reporting | Selective reporting | Low | No evidence of selective reporting was observed. |
| | Other | | Low | No other sources of potential bias were identified. |

**Table 1.5 Risk of bias amongst individual pregnancy studies (continued)**

| Reference | Type of bias | Description | Risk | Support for judgement of bias |
|---|---|---|---|---|
| Ramharter (2007) [178] | Selection | Random sequence generation | Unclear | Pregnant women attending health facilities for delivery at two different sites were invited to participate. General demographic data was presented for 2004 and 2006, but not stratified by IPTp dosing and gravidae, making it difficult to determine whether the selection process introduced a systematic bias. |
| | Selection | Allocation concealment | Unclear | It is unclear whether the lack of allocation concealment introduced selection bias. |
| | Performance | Blinding of participants and personnel | Low | Although participants and personnel were not blinded, newborn birth weights were measured to the nearest 5 g immediately after delivery. |
| | Detection | Blinding of outcome assessment | Unclear | It is unclear if analysis of birth weight outcomes and exposure to IPTp-SP was blinded. |
| | Attrition | Incomplete outcome data | Unclear | Amongst 389 women in 2004, birth weights were reported for 385 newborns (99.0%); amongst 1,014 women in 2006, birth weights were reported for 788 newborns (77.7%). |
| | Reporting | Selective reporting | Unclear | Comparison of data with historical controls may be affected by bias and confounding. |
| | Other | | Unclear | One hundred thirty-one of 389 (33.7%) women reported regular chemoprophylaxis with chloroquine during pregnancy in survey 2004. To what extent this affected the outcomes of controls is unclear. |

**Table 1.5 Risk of bias amongst individual pregnancy studies (continued)**

| Reference | Type of bias | Description | Risk | Support for judgement of bias |
|---|---|---|---|---|
| Rogerson (2000) [182] | Selection | Random sequence generation | Unclear | Pregnant women in active labour, and likely to deliver during working hours, were asked to participate in the study. From this group, data related to IPTp-SP exposure was collected. Although education on schooling was available for 50.4% of participants (526/1,044), demographic data were limited for comparing differences amongst women. |
| | Selection | Allocation concealment | Unclear | Allocation was based on prior exposure to IPTp-SP; it is unclear if this may have introduced any bias into the analysis. |
| | Performance | Blinding of participants and personnel | Unclear | Method and timing of weighing newborns was not reported. |
| | Detection | Blinding of outcome assessment | Unclear | It is unclear if analysis of birth weight outcomes and exposure to IPTp-SP was blinded. |
| | Attrition | Incomplete outcome data | Low | There appears to be no evidence of incomplete outcome data. |
| | Reporting | Selective reporting | Low | There is no evidence of selective reporting |
| | Other | | Low | No apparent risk of other bias. |

**Table 1.5 Risk of bias amongst individual pregnancy studies (continued)**

| Reference | Type of bias | Description | Risk | Support for judgement of bias |
|---|---|---|---|---|
| Sirima (2006) [180] | Selection | Random sequence generation | Unclear | Pregnant women (n=826) from six antenatal care facilities and from two delivery units (n=1,188) were enrolled. Because 20 eligible women (2.4%) refused enrolment at antenatal care facilities and 33 (2.8%) at delivery units, selection bias is likely low amongst recipients of SP. However, it is unclear if and to what extent bias was introduced when comparing these women to who did not receive SP and demographic data did not appear to be used to control for potential differences. |
| | Selection | Allocation concealment | Unclear | It is unclear whether the lack of allocation concealment introduced selection bias. |
| | Performance | Blinding of participants and personnel | Low | Neonates were weighed to the nearest 10 grams using an electronic digital scale. |
| | Detection | Blinding of outcome assessment | Low | Although it is unknown whether outcome assessment was blinded, clear and consistent gravidae-specific trends were associated with malaria infection as well as a dose-response relationship between SP and low birth weight. |
| | Attrition | Incomplete outcome data | Low | Amongst enrolled women, 97.4% had a baby whose birth weight was measured. |
| | Reporting | Selective reporting | Low | There is no evidence of selective reporting. |
| | Other | | Unclear | An unknown number of pregnant women took IPTp-SP doses at home rather than ingesting them while observed in at the health facility; it is possible that not all of these doses were actually taken as reported. |

**Table 1.5 Risk of bias amongst individual pregnancy studies (continued)**

| Reference | Type of bias | Description | Risk | Support for judgement of bias |
|---|---|---|---|---|
| van Eijk (2004) [179] | Selection | Random sequence generation | Unclear | All women were invited to participate who received ANC services at the study hospital and delivered at the same facility between June 1999 and June 2000. The number of SP doses on the ANC card was confirmed with the mother. |
| | Selection | Allocation concealment | Unclear | See above |
| | Performance | Blinding of participants and personnel | Unclear | Methods of neonatal weighing and post-partum timing of weighing were not reported. |
| | Detection | Blinding of outcome assessment | Unclear | It is unclear if analysis of low birth weight was conducted in a blinded manner. |
| | Attrition | Incomplete outcome data | Low | Only women who delivered in the hospital, and had previously visited the ANC of the hospital, as well as complete information on their birth card (n=1,498) were included in the analysis, representing 89.5% of eligible women. |
| | Reporting | Selective reporting | Low | No selective reporting observed |
| | Other | | Low | No apparent risk. |

**Figure 1.4 Risk of bias amongst individual pregnancy studies**

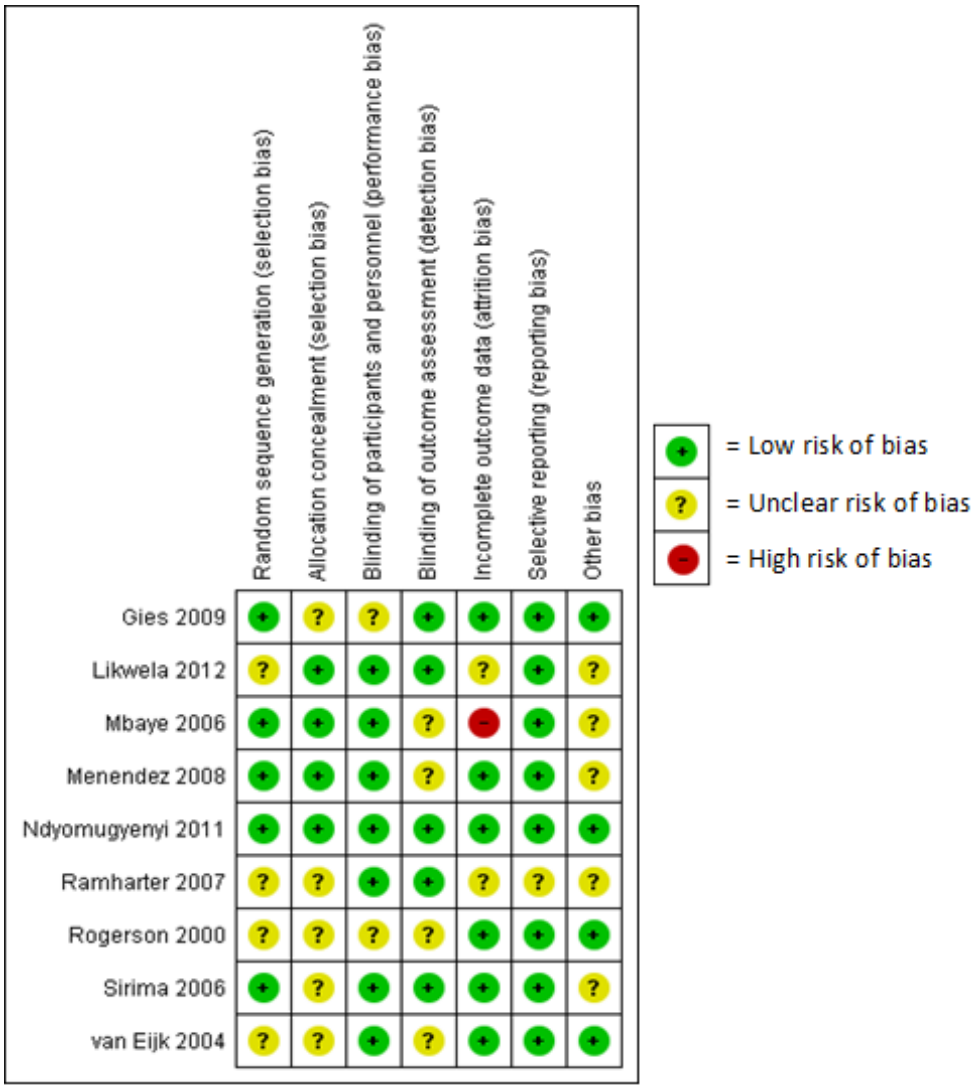
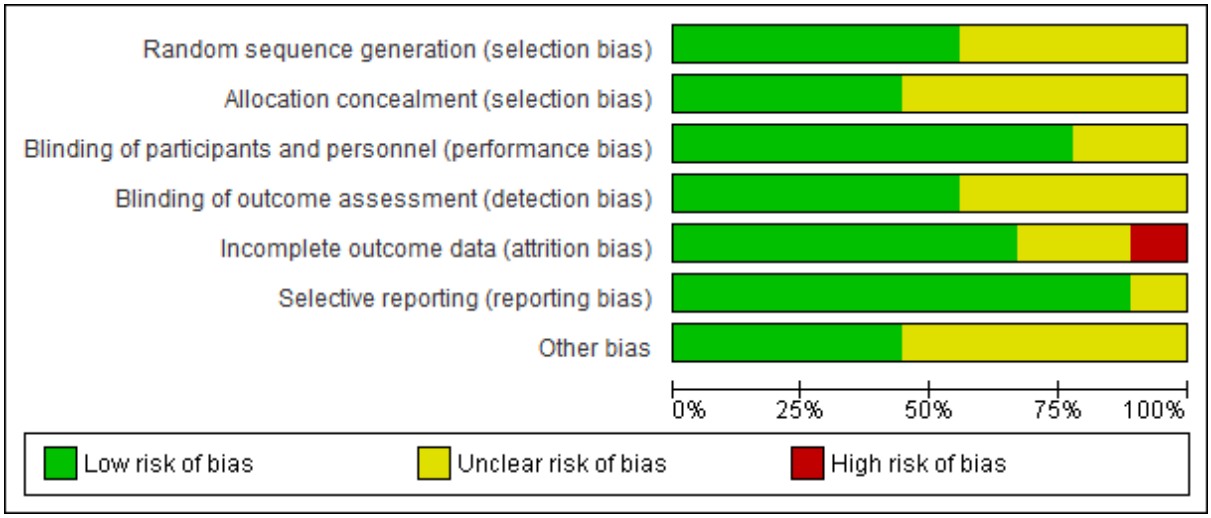



**Figure 1.5 Risk of bias across pregnancy studies**



**Meta-analysis**

There was strong evidence of protection against LBW conferred by $\geq$ 2 doses versus placebo or no doses of IPTp-SP. Random-effects models showed that amongst primi- and secundigravidae $\geq$ 2 doses cut the odds in half of delivering a LBW newborn (odds ratio [OR] = 0.54; 95% CI: 0.35, 0.84; $P < 0.00$) compared to no doses or placebo. The odds of LBW were reduced by 30% amongst multigravidae (OR = 0.70; 95% CI: 0.51, 0.95; $P = 0.04$).

In order to estimate the transmission intensity at each of the IPTp-SP study sites, 12 national cross-sectional datasets were used from MAP containing 11,548 surveys. Pooled prevalence estimates were calculated as a second means of estimating the malaria transmission intensity at 10 sites under rule 1, and for eight sites by applying rule 3. The remaining four study sites were matched based on rule 4. Matching by gravidae was as follows: (i) six of 12 primi- and secundigravidae IPTp-SP study sites were matched under rule 1; (ii) three study sites were paired under rule 3; (iii) three study sites were matched using rule 4. Amongst studies with multigravidae, four were matched using rule 1, five were paired under rule 3, and one study site was matched based on rule 4. As shown in Table 1.6, parasite prevalence amongst primi- and secundigravidae derived from MAP estimates were between 20.5% and 66.4%, whereas the pooled prevalence estimates were slightly narrower, 25.1% and 59.1%. Amongst multigravidae, MAP estimates were between 20.5% and 66.4%, and the pooled prevalence estimates were also narrower from 6.7% and 39.3%.

**Table 1.6 Malaria transmission estimates based on matching rules**

| References | Country | Site | Elevation | Rule used | Pooled prevalence (%) | 2007 *Pf*PR$_{2-10}$ (%) |
|---|---|---|---|---|---|---|
| **Primi- and secundigravidae** | | | | | | |
| Likwela (2012) [176] | DR Congo | Rutshuru | 1212 | 4 | 29.6 [a] | 29.6 |
| Ndyomugyenyi (2011)[*] [81] | Uganda | Kabale | 2118 | 4 | 25.1 [a] | 25.1 |
| Ndyomugyenyi (2011)[†] [81] | Uganda | Kabale | 2118 | 4 | 25.1 [a] | 25.1 |
| Likwela (2012) [176] | DR Congo | Kisangani | 401 | 1 | 30.4 | 40.2 |
| Menendez (2008) [77] | Mozambique | Manhiça | 21 | 1 | 31.2 | 48.1 |
| Gies (2009) [177] | Burkina Faso | Boromo | 258 | 1 | 44.2 | 57.4 |
| Likwela (2012) [176] | DR Congo | Mikalayi | 618 | 3 | 55.8 | 38.5 |
| Ramharter (2007) [178] | Gabon | Libreville | 13 | 3 | 55.8 | 37.1 |
| Ramharter (2007) [178] | Gabon | Lambaréné | 39 | 3 | 55.8 | 42.8 |
| van Eijk (2004) [179] | Kenya | Kisumu | 1166 | 1 | 57.5 | 20.5 |
| Sirima (2006)[*] [180] | Burkina Faso | Koupéla | 290 | 1 | 59.1 | 66.4 |
| Sirima (2006)[†] [180] | Burkina Faso | Koupéla | 290 | 1 | 59.1 | 66.4 |
| **Multigravidae** | | | | | | |
| Ndyomugyenyi (2011)[‡] [81] | Uganda | Kabale | 2118 | 3 | 6.7 | 25.1 |
| Ndyomugyenyi (2011)[§] [81] | Uganda | Kabale | 2118 | 3 | 6.7 | 25.1 |
| Mbaye (2006) [80] | The Gambia | Farafenni | 25 | 1 | 8.3 | 23.9 |
| Rogerson (2000) [181] | Malawi | Blantyre | 989 | 3 | 11.3 | 24.9 |
| Menendez (2008)[¶] [77] | Mozambique | Manhiça | 21 | 3 | 11.3 | 48.1 |
| van Eijk (2004) [179] | Kenya | Kisumu | 1166 | 1 | 25.0 | 20.5 |
| Likwela (2012) [176] | DR Congo | Rutshuru | 1212 | 4 | 29.6 [a] | 29.6 |
| Sirima (2006) [180] | Burkina Faso | Koupéla | 290 | 1 | 32.9 | 66.4 |
| Likwela (2012) [176] | DR Congo | Kisangani | 401 | 3 | 34.0 | 40.2 |
| Likwela (2012) [176] | DR Congo | Mikalayi | 618 | 1 | 39.3 | 38.5 |

Note: Studies are presented in ascending order of malaria transmission intensity; [a] Under rules 1-3, no matches were made and, therefore, the 2007 *Pf*PR$_{2-10}$ estimates from MAP are used. [*] = primigravidae; [†] = secundigravidae; [‡] = 2-4 pregnancies; [§] = 5 or more pregnancies; [¶] = 1-3 pregnancies (likely contained some secundigravidae) and 4 or more pregnancies

**Meta-regression analysis**

To explore the potential effect of malaria transmission intensity on the association between IPTp-SP and LBW, four separate meta-regression models were conducted. Models one and two applied the odds ratios of protection conferred by $\geq$ 2 doses of IPTp-SP versus placebo or no doses amongst primi- and secundigravidae with model one employing transmission intensity estimates from MAP, and model two using pooled prevalence estimates for transmission intensity. Models three and four were constructed using the odds ratios from analyses of multigravidae and transmission intensity estimates from MAP and, separately, pooled prevalence estimates derived from cross-sectional surveys. Not one of these four models suggested that variation in the ORs of LBW could be explained by malaria transmission intensity: model one ($P$ = 0.83); model two ($P$ = 0.78); model three ($P$ = 0.30); model four ($P$ = 0.93). Sensitivity testing amongst matching rules did not produce any statistically significant difference in these results.

## Discussion

This analysis demonstrates that there is no level of malaria transmission intensity below which $\geq$ 2 doses of IPTp-SP no longer protects against the incidence of LBW. The inability of this study to detect a malaria transmission threshold may be a consequence of there being too few data points for analysis, particularly from lower transmission settings. Data from Ndyomugyenyi *et al.* in Uganda complicates interpretation because women in the no-SP group were provided ITNs. Consequently, the difference between women in the 'protected' versus 'unprotected' groups was likely harder to detect because there was less total exposure to malaria infection rather than IPTp-SP being compromised. However, an unpublished analysis of

Multiple Indicator Cluster Surveys with several thousand data points also found that IPTp-SP continued to protect against LBW until very low levels of transmission and, similar to this study, could not define a cut-off point of transmission below which IPTp-SP is no longer protective against LBW (Eisele TP, personal communication).

There may be other factors that have contributed to not being able to detect a transmission threshold. Little is known about the prevalence of placental infection in very low transmission settings and it is entirely possible that declines in peripheral parasitaemia are not reflected in equivalent reductions in placental infection. If that is the case, then IPTp-SP may provide important and continued protection against placental carriage, as well as the incidence of LBW in areas of very low endemicity. Another possible explanation is that the causal pathway to LBW is multifactorial and that IPTp-SP offers some protection against other non-malarial causes of LBW. Indeed, this is what was observed in the study conducted under Q 3. Although not common, SP can be used as an efficacious chemoprophylactic drug against *Pneumocystis jiroveci* pneumonia and *Toxoplasma gondii* infection.[155] Moreover, sulphadoxine is related to sulphamethoxazole, the partner compound used with trimethoprim to form cotrimoxazole, a treatment often provided to cure urinary tract infections and to prevent *P. jiroveci* in HIV-infected patients.[156] Sulphonamides have also been used to treat *Gardnerella vaginalis*,[159] a bacterium found in women with bacterial vaginosis that can double the odds of having a low birth weight baby (OR = 2.0; 95% CI: 1.3, 2.9) compared to pregnant women without bacterial vaginosis.[12]

The MAP *Pf*PR$_{2-10}$ estimates from 2007 were not the best temporal match for the pregnancy studies; all but one of the studies occurred before 2007. However, individual survey data available through MAP did produce more precise estimates of parasitaemia with narrower confidence intervals. However, these data only contained the age range of participants from whom samples had been collected. Thus, it was sometimes necessary to make assumptions about which age group to assign survey data, i.e. 0 to > 5 years of age to primi- and secundigravidae versus 5-15 years of age to multigravidae, to create a binomial structure of data that might mirror the parity-specific epidemiology of malaria in pregnancy.

The results from this analysis should be interpreted with caution for several reasons. This analysis has been conducted with the limited data available that relate the efficacy of IPTp-SP to the intensity of malaria exposure during pregnancy and that can also be stratified by gravidae. Moreover, there may have been selection bias in six of the nine studies identified through systematic review for lack of random sequence generation or allocation concealment. In addition, there was considerable heterogeneity amongst IPTp-SP studies, making it more difficult to generalise about them as a group. However, stratifying studies by design, observational versus RCT, did not consistently reduce heterogeneity, nor change the observed results.

## Q 2. A581G prevalence threshold

### Methods

The data set of IPTp-SP studies developed to answer the first research question was used as the starting point to estimate the prevalence threshold of the *Pfdhps* mutation

at codon A581G above which IPTp-SP may no longer protect against the incidence of LBW. A geographical database of SP resistance markers[183], originally developed to guide policy recommendations specific to intermittent preventive treatment of malaria using SP amongst African infants, was used to identify surveys containing point prevalence estimates of the A581G mutation that had been collected from < 100 miles and plus or minus two years of the IPTp-SP trials. The geographic range was extended 100 to 250 miles if no data were available from < 100 miles. If multiple point estimates were found, then data were pooled using standard meta-analysis.

## Results

Point prevalence estimates of *Pfdhps* A581G were identified from 44 sites having been measured within plus or minus two years of when the IPTp-SP trials had been conducted. Twenty sites were within < 100 miles of IPTp-SP trials and 24 sites within 100 to 250 miles. All IPTp-SP trial sites had at least one corresponding point estimate of A581G as shown in Table 2.1 between these two distance parameters.[184-198] The highest prevalence of A581G was 52.6% (95% CI: 47.6, 57.5) based on 740 samples as measured at four sites < 100 miles of the IPTp-SP study in Kabale (Uganda). Prevalence estimates for the other sites ranged from 0.0% to 10.1% (95% CI: 3.5, 16.8).

There was a binomial structure in the A581G prevalence data that lent itself to stratification of IPTp-SP studies where there A581G prevalence of $\leq$ 10.1% and > 10.1%. Based on random-effects models, $\geq$ 2 doses of IPTp-SP reduced the odds of LBW amongst primi- and secundigravidae (OR = 0.49; 95% CI: 0.29, 0.81; $P < 0.00$) and multigravidae (OR = 0.56; 95% CI: 0.37, 0.86; $P < 0.03$) in areas where there A581G prevalence was $\leq$ 10.1%. Two or more doses of IPTp-SP continued to protect primi-

and secundigravidae against LBW (OR = 0.54; 95% CI: 0.35, 0.84). This protective effect was no longer statistically significant in areas where A581G prevalence was > 10.1% (OR = 0.73; 95% CI: 0.29, 1.18). Where are also geographic areas where malaria transmission intensity was lowest according to pooled and MAP prevalence estimates (range: 25.1% to 29.6%). Amongst multigravidae, IPTp-SP conferred protection against LBW in areas where the prevalence of A581G was $\leq$ 10.1% (OR = 0.56; 95% CI: 0.37, 0.86; *P* = 0.07). However, there was no evidence from fixed-effects or random-effects models that multigravidae continued to be protected against LBW where the prevalence of A581G was > 10.1% (OR = 0.96; 95% CI: 0.70, 1.34; *P* < 0.47). These results are illustrated in Figures 2.1 and 2.2. The interaction between malaria transmission intensity and the prevalence of A581G was borderline significant: *P* = 0.06 amongst primi- and secundigravidae, and *P* = 0.04 amongst multigravidae. Thus, meta-regression of malaria transmission, stratified by A581G prevalence, could only explain some variation in IPTp-SP protection against LBW.

## Table 2.1 A581G Prevalence at IPTp-SP study sites

| Pregnancy studies | | | *Pf*dhps codon A581G surveys | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **References** | **Countries** | **Sites** | **Distance from IPTp-SP trial** | **No. sites** | **No. tested** | **No. positive** | **Prevalence (%)** | **95% CI** | **Heterogeneity ($I^2$)** | **Prevalence across sites (%)** | **Refs** |
| Gies (2009) | Burkina Faso | Boromo | 100-250 m | 3 | 250 | 26 | 1.7* | 0.2 to 3.2 | 93.8% | 0.0 to 37.8 | 184-186 |
| Likwela (2012) | DR Congo | Kisangani | 100-250 m | 1 | 18 | 1 | 5.6 | -5.0 to 16.1 | NA | NA | 187 |
| Likwela (2012) | DR Congo | Mikalayi | 100-250 m | 0 | 0 | 0 | 0.0 | NA | NA | NA | 187 |
| Likwela (2012) | DR Congo | Rutshuru | < 100 m | 6 | 747 | 367 | 52.4 [1] | 47.5 to 57.4 | 79.6% | 28.6 to 61.1 | 187-190 |
| Mbaye (2006) | The Gambia | Farafenni | < 100 m | 1 | 27 | 0 | 0.0 [1] | NA | NA | 0.0 | 191 |
| Menendez (2008) | Mozambique | Manhiça | 100-250 m | 4 | 279 | 0 | 0.0 [1] | NA | NA | 0.0 | 192 |
| Ndyomugyenyi (2011) | Uganda | Kabale | < 100 m | 5 | 740 | 365 | 52.6 | 47.6 to 57.5 | 83.3% | 30.2 to 61.1 | 187-190 |
| Ramharter (2007) | Gabon | Lambaréné | 100-250 m | 2 | 118 | 0 | 0.0 | NA | NA | 0.0 | 193,194 |
| Ramharter (2007) | Gabon | Libreville | 100-250 m | 2 | 236 | 4 | 2.0 [2] | 0.06 to 3.9 [2] | NA | 0.0 to 2.0 | 11,12 |
| Rogerson (2000) | Malawi | Blantyre | < 100 m | 3 | 318 | 3 | 3.4 [2] | -0.4 to 7.1 [2] | 100% | 0.0 to 3.4 | 195,196 |
| Sirima (2006) | Burkina Faso | Koupéla | < 100 m | 1 | 79 | 8 | 10.1 [2] | 3.5 to 16.8 [2] | NA | 10.1 | 186 |
| van Eijk (2004) | Kenya | Kisumu | < 100 m | 6 | 825 | 4 | 2.7† | 0.1 to 5.4 [2] | 100% | 0.0 to 2.7 | 197,198 |

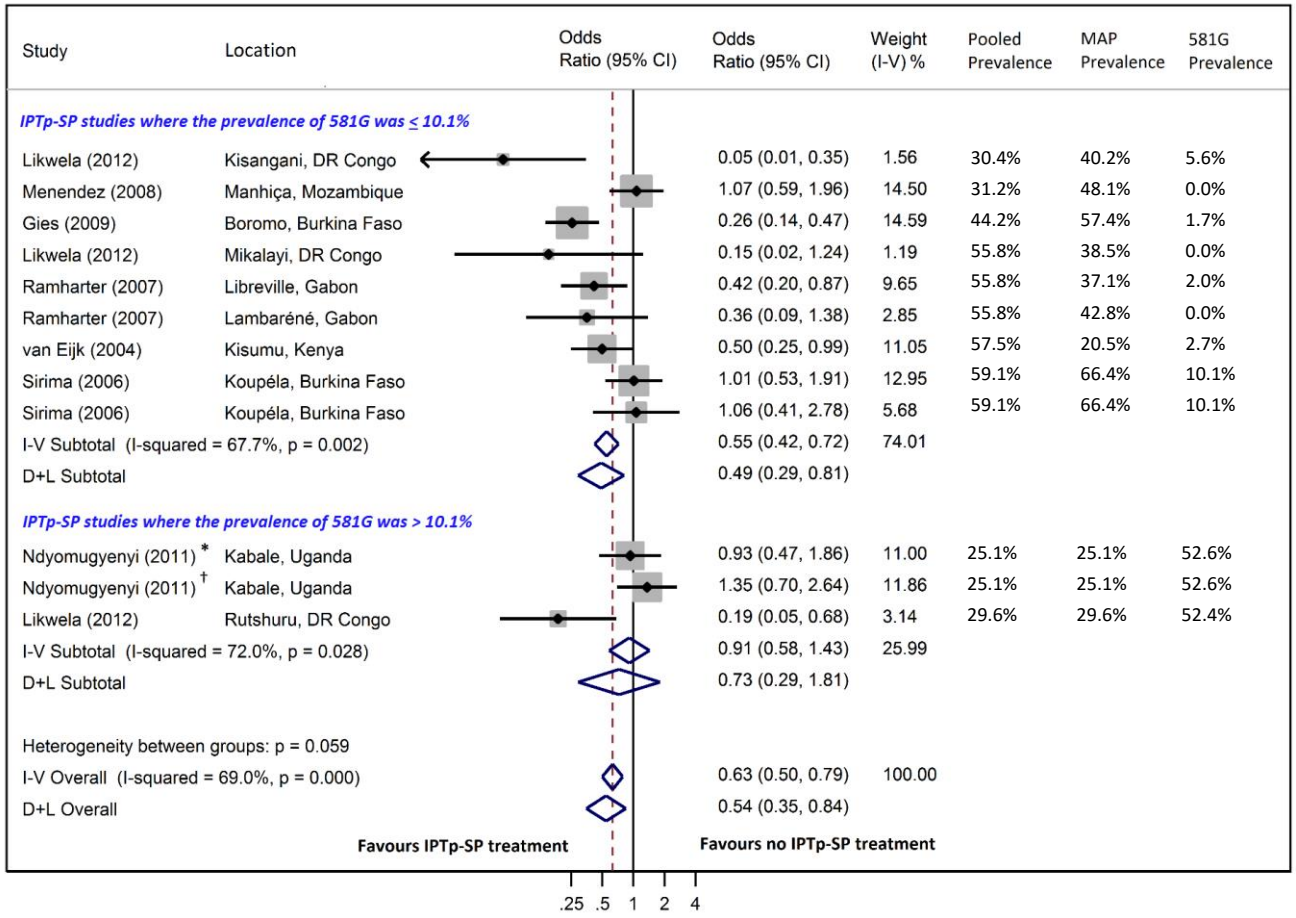
NA = Not applicable

[1] No mutations were detected and, therefore, the prevalence shown is the point estimate of the one study that did yield positive samples

[2] Only one of the studies detected mutations; models exclude studies in which there are no positive tests and, therefore, the prevalence shown is a point estimate of the one study that did yield positive samples.

**Figure 2.1 Forest plot – primi- and secundigravidae**

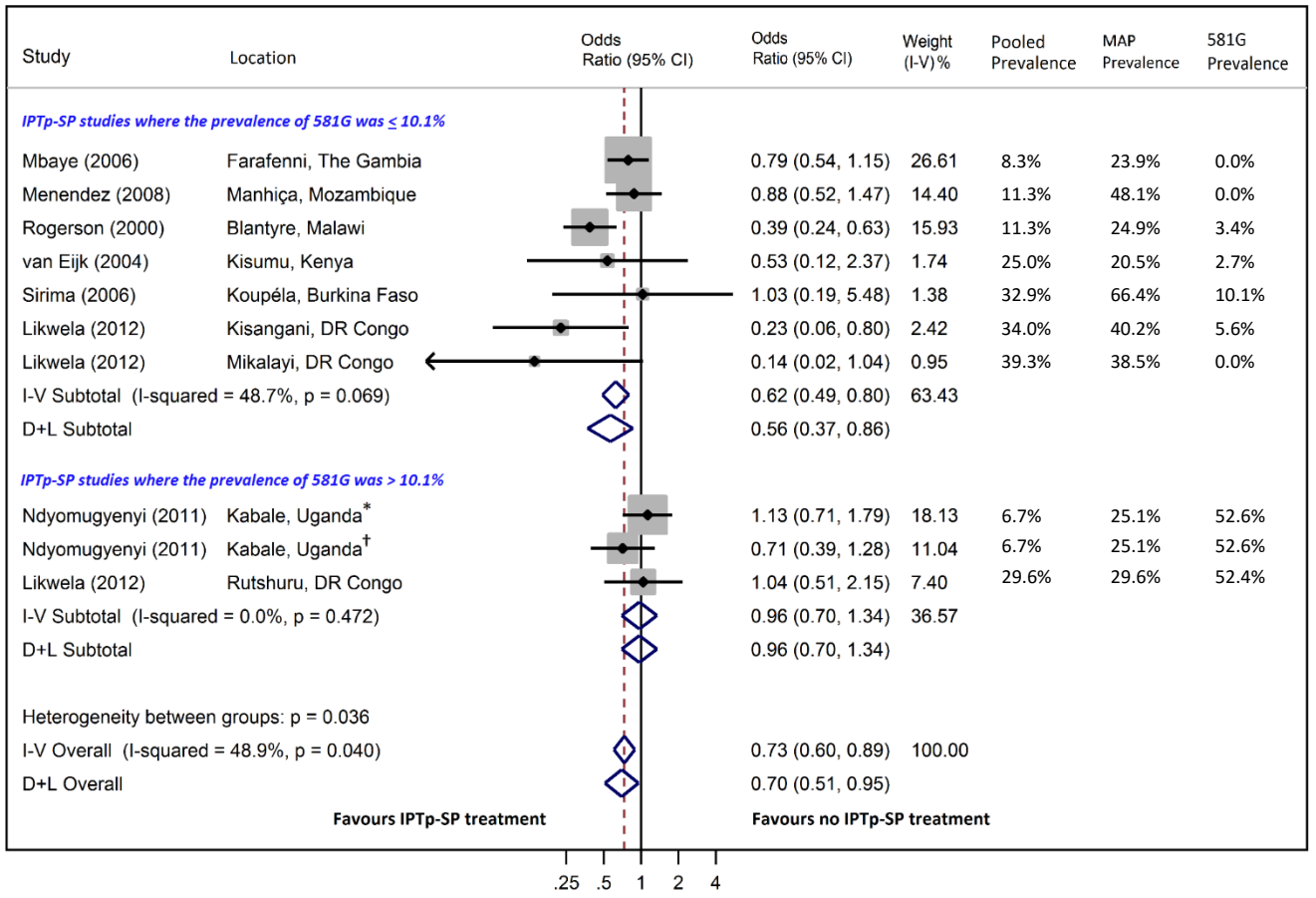
Odds ratio of low birth-weight delivery amongst primi- and secundigravidae following two or more doses of IPTp-SP versus placebo or no IPTp-SP stratified by estimates of the A581G resistance mutation amongst malaria parasites at study sites




Note: Studies are presented in ascending order of malaria transmission intensity * Secundigravidae; † Primigravidae; Vertical red line (dashed) = Pooled odds ratio; I–V overall: fixed-effects estimate; D + L overall**:** random-effects estimate; Diamond shapes = Pooled estimate and 95% CIs

**Figure 2.2 Forest plot – multigravidae**

Odds ratio of low birth-weight delivery amongst multigravidae following two or more doses of IPTp-SP versus placebo or no IPTp-SP stratified by estimates of the A581G resistance mutation amongst malaria parasites at study sites




Note: Studies are presented in ascending order of malaria transmission intensity; * = 2-4 pregnancies; † = 5 or more pregnancies; Vertical red line (dashed) = Pooled odds ratio; I–V overall: fixed-effects estimate; D + L overall**:** random-effects estimate; Diamond shapes = Pooled estimate and 95% CIs

## Discussion

In this analysis IPTp-SP studies were stratified by primi- and secundigravidae versus multigravidae, and the effect that the A581G mutation on protection conferred against LBW was investigated. This biomarker-based measure of SP resistance provides some clarity on where, geographically, a replacement for SP might be appropriate amongst multigravidae.

Given the lack of prevalence estimates for the A581G mutation between 10.1% and 52.4%, it is only reasonable to conclude that the prevalence threshold of this mutation is > 10.1% above which IPTp-SP no longer protects against the incidence of LBW amongst multigravidae. It may be tempting to state the cut-off should be > 52.4%, or a convenient level such as 50%. The actual threshold may be much lower, however, especially considering the alarming results from Muheza, Tanzania, where researchers found placental infection significantly higher in 84% of women (n = 104) who were given any IPTp-SP compared to 16% of women (n = 104) who received no doses of IPTp-SP ($P = 0.03$).[86,199] The prevalence of the A581G mutation in this study area was 55% (95% CI: 44.7, 65.2; N = 87).[88]

The results from this analysis should be interpreted with caution for the same reasons noted in the investigation of transmission intensity. This analysis has been conducted with the limited data available. As previously mentioned, there may have been selection bias in six of the nine studies identified through systematic review for lack of random sequence generation or allocation concealment. In addition, there was considerable heterogeneity amongst IPTp-SP studies, making it more difficult to generalise about them as a group. However, stratifying studies by design did not consistently reduce heterogeneity, nor change the results.

The A581G mutation is not widely found throughout sub-Saharan Africa, although the codon has been detected in 10 countries[152] and the codon can be selected rapidly. A study in Kenya reported the prevalence of A581G to be 85.1% (95% CI: 80.0%, 89.4%) where no parasites had expressed the mutation when surveillance testing was conducted three years earlier.[200] However, before declaring a specific level of malaria transmission or A581G prevalence at which IPTp-SP no longer provides a cost-effective benefit, placebo-control trials may be need in a range of low-transmission settings that are sufficiently powered for sub-analysis by gravidae.

## Q 3. Effect of IPTp-SP on malaria and curable STIs/RTIs in pregnancy

### Methods

To estimate the effect of IPTp-SP on malaria infection and curable STIs/RTIs in pregnancy, a secondary analysis was conducted using data collected for a prospective cohort study of the burden of malaria and curable STIs/RTIs carried out between November 2013 and April 2014 in the Nchelenge District of Zambia.[201,202] Nchelenge is in the Luapula province, 290 km (180 miles) from Mansa, the provincial capital, and along the shores of Lake Mweru in north-east Zambia. In total, 1,086 pregnant women were recruited on their first ANC visit to health centres operated by the Zambian Ministry of Health. As part of determining eligibility for participation, women had their gestational age calculated according to their last menstrual period, symphysis-fundal height measurement, and sonography. Women who were not already known to have the AIDS virus were tested for HIV per Ministry practices. HIV-infected women were given triple antiretroviral therapy if their CD4 count dropped below 500 cells per cubic millimetre of blood, representing a variation on 'Option B' treatment originally

recommended by the WHO in 2010.[203] Study staff tested women for syphilis using rapid plasma reagin (RPR) methods and, if positive, the results were sent to participants with a notice to return to the health facility with their partners for treatment. Biological samples for malaria and other curable STIs/RTIs were also collected at enrolment and transported to a reference laboratory for retrospective batch testing where PCR techniques were used. At delivery, placental samples were collected for malaria histology. During scheduled ANC visits, women received IPTp-SP and were followed by study staff until delivery to record birth outcomes. Women were encouraged to deliver at a health facility for maximum care and to ease data collection. If willing to do so, women were provided free transport to and from the only hospital in the district, Saint Paul's Mission Hospital, or Kashikishi Health Centre. At the time, Nchelenge Health Centre did not offer maternity services. Data were analysed using Stata version 13 (Stata Corp, College Station, Texas) software.

***Descriptive analysis***

Categorical variables were summarised as whole numbers and percentages, whereas continuous variables were described by means and standard deviations if the data appeared to be normally distributed. Differences in the characteristics of participants at the time of enrolment were compared between women who had received 0 to 1 dose and $\geq$ 2 doses of IPTp-SP and, separately, 2 doses versus $\geq$ 3 doses, using Wilcoxon test for continuous variables and Fisher's exact test for categorical variables. These variables are listed in Table 3.6. Crude odds ratios were then calculated of adverse birth outcomes amongst pregnant women who received 0 to 1 dose

compared ≥ 2 doses and, separately, 2 doses compared to ≥ 3 doses using logistic regression models.

***Assessment of potential confounding and effect modification factors***

HIV status and gravidae were considered to confound the protective effect of IPTp-SP on birth outcomes based on studies from the literature.[204,205] Common statistical methods were applied to identify other potential confounders,[206] entering variables into a logistic regression model that included categories of doses of IPTp-SP exposure. Variables were considered *a priori* to be confounders if they produced a change in the odds ratios of crude versus adjusted by 10% or more as shown in Tables 3.1 to 3.5. Effect modification was tested by adding an interaction term between these potential confounders and the number of IPTp-SP doses, and then applying a likelihood ratio test to determine whether there was evidence of interaction. Some evidence of effect modification with malaria and STI/RTI co-infection was observed which is dealt with separately below. The co-infection of malaria and STIs/RTIs was also considered a confounder. All confounding variables were entered in a multivariable logistic regression model to estimate overall adjusted odds ratios.

***Assessment of association between IPTp-SP and maternal malaria and STIs/RTIs***

Seven mutually exclusive maternal infection categories were constructed to examine the distribution of mono- and co-infection: (i) malaria only, (ii) malaria and *N. gonorrhoeae* and/or *C. trachomatis*, (iii) malaria and *T. vaginalis* and/or bacterial vaginosis, (iv) syphilis and any other infection(s), (v) *N. gonorrhoeae, C. trachomatis* only, (vi) *T. vaginalis* and/or bacterial vaginosis only, and (vii) no identified infection.

Odds of IPTp-SP exposure to 0-1 dose versus $\geq$ 2 doses of IPTp-SP were calculated for each infection category by adverse birth outcome using a multivariable logistic regression model that included all potential confounders and interaction terms.

## Table 3.1 Potential confounding factors for any adverse birth outcome

| Potential confounding variable | Crude analysis | | | Adjusted analysis | | | % change in crude odds ratio[5] | *P*-value for homogeneity | Missing values[6] |
|---|---|---|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | *P*-value[4] | Odds ratio | 95% CI | *P*-value[4] | | | |
| Gravidae | 0.57 | (0.38, 0.84) | 0.004 | 0.52 | (0.35, 0.77) | 0.001 | 8.89 | 0.302 | 0 |
| Prior preterm birth[1] | 0.42 | (0.15, 1.19) | 0.092 | 0.38 | (0.13, 1.11) | 0.067 | 7.88 | * | 620 |
| Sex of baby | 0.57 | (0.38, 0.84) | 0.004 | 0.59 | (0.40, 0.87) | 0.007 | 3.86 | 0.240 | 0 |
| Co-infection (malaria and/or STI/RTI) | 0.57 | (0.38, 0.84) | 0.004 | 0.54 | (0.37, 0.81) | 0.002 | 3.81 | 0.125 | 0 |
| Maternal age at enrolment (years) | 0.57 | (0.38, 0.84) | 0.004 | 0.55 | (0.36, 0.82) | 0.003 | 3.55 | 0.736 | 0 |
| Prior miscarriage[1] | 0.42 | (0.15, 1.19) | 0.092 | 0.40 | (0.14, 1.15) | 0.079 | 3.39 | 0.424 | 620 |
| Placental malaria (PCR diagnosis) | 0.58 | (0.39, 0.85) | 0.005 | 0.56 | (0.38, 0.83) | 0.004 | 2.63 | 0.853 | 7 |
| Delivery type | 0.57 | (0.38, 0.84) | 0.004 | 0.58 | (0.39, 0.86) | 0.006 | 2.38 | 0.929 | 0 |
| Treatment of malaria infection during pregnancy[2] | 0.58 | (0.39, 0.86) | 0.006 | 0.57 | (0.38, 0.84) | 0.004 | 2.23 | 0.256 | 3 |
| Marital status | 0.57 | (0.38, 0.84) | 0.004 | 0.55 | (0.38, 0.81) | 0.002 | 2.22 | 0.001 | 0 |
| Number of lifetime sexual partners | 0.56 | (0.38, 0.83) | 0.004 | 0.57 | (0.39, 0.85) | 0.005 | 2.06 | 0.564 | 6 |
| Hypertension at enrolment or delivery | 0.54 | (0.36, 0.82) | 0.003 | 0.55 | (0.37, 0.83) | 0.004 | 1.93 | 0.213 | 86 |
| Type of personnel attending birth | 0.57 | (0.38, 0.84) | 0.004 | 0.58 | (0.39, 0.85) | 0.005 | 1.78 | 0.042 | 0 |
| HIV status | 0.57 | (0.38, 0.84) | 0.004 | 0.57 | (0.39, 0.85) | 0.005 | 1.42 | 0.946 | 0 |
| Age of sexual debut (years) | 0.57 | (0.38, 0.84) | 0.004 | 0.57 | (0.39, 0.85) | 0.005 | 1.38 | 0.243 | 0 |
| Prior stillbirth[1] | 0.42 | (0.15, 1.19) | 0.092 | 0.42 | (0.14, 1.25) | 0.108 | 1.33 | 0.358 | 620 |
| Indoor residual spraying in preceding 12 months | 0.53 | (0.36, 0.80) | 0.002 | 0.53 | (0.35, 0.79) | 0.001 | 1.30 | 0.896 | 26 |
| Syphilis at enrolment (high titre) | 0.57 | (0.38, 0.84) | 0.004 | 0.56 | (0.38, 0.83) | 0.004 | 1.16 | 0.400 | 5 |
| Labour type | 0.57 | (0.39, 0.85) | 0.005 | 0.57 | (0.38, 0.84) | 0.004 | 1.14 | * | 15 |
| Bed net ownership | 0.57 | (0.38, 0.84) | 0.004 | 0.57 | (0.39, 0.85) | 0.005 | 1.10 | 0.750 | 0 |
| Bed net usage (on night prior to survey) | 0.56 | (0.38, 0.83) | 0.004 | 0.55 | (0.37, 0.82) | 0.003 | 1.09 | 0.826 | 3 |
| Delivery location | 0.57 | (0.38, 0.84) | 0.004 | 0.57 | (0.38, 0.85) | 0.005 | 1.05 | 0.070 | 0 |
| *T. vaginalis* co-infection (malaria and/or STI/RTI) | 0.57 | (0.38, 0.84) | 0.004 | 0.57 | (0.39, 0.84) | 0.004 | 0.89 | 0.011 | 0 |

## Table 3.1 Potential confounding factors for any adverse birth outcome (continued)

| Potential confounding variable | Crude analysis | | | Adjusted analysis | | | % change in crude odds ratio[5] | *P*-value for homogeneity | Missing values[6] |
|---|---|---|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | *P*-value[4] | Odds ratio | 95% CI | *P*-value[4] | | | |
| Wealth quintiles | 0.57 | (0.38, 0.84) | 0.004 | 0.56 | (0.38, 0.83) | 0.004 | 0.84 | 0.503 | 0 |
| Maternal haemoglobin level at delivery[3] | 0.60 | (0.40, 0.90) | 0.012 | 0.60 | (0.40, 0.90) | 0.012 | 0.56 | 0.101 | 32 |
| Treatment of STIs/RTIs during pregnancy including syphilis | 0.76 | (0.46, 1.23) | 0.259 | 0.76 | (0.47, 1.23) | 0.265 | 0.49 | 0.250 | 258 |
| STI/RTI co-infection | 0.57 | (0.38, 0.84) | 0.004 | 0.57 | (0.38, 0.84) | 0.004 | 0.39 | 0.483 | 5 |
| *N. gonorrhoeae* co-infection (malaria and/or STI/RTI) | 0.57 | (0.38, 0.84) | 0.004 | 0.56 | (0.38, 0.84) | 0.004 | 0.39 | 0.318 | 0 |
| Bacterial vaginosis and STI co-infection | 0.57 | (0.38, 0.84) | 0.004 | 0.57 | (0.39, 0.84) | 0.004 | 0.33 | 0.067 | 5 |
| Recruitment site | 0.57 | (0.38, 0.84) | 0.004 | 0.56 | (0.38, 0.84) | 0.004 | 0.24 | 0.991 | 0 |
| Treatment of STIs/RTIs during pregnancy excluding syphilis | 0.57 | (0.38, 0.84) | 0.004 | 0.57 | (0.38, 0.84) | 0.004 | 0.19 | 0.492 | 0 |
| *C. trachomatis* co-infection (malaria or STI/RTI) | 0.57 | (0.38, 0.84) | 0.004 | 0.57 | (0.38, 0.84) | 0.004 | 0.04 | 0.770 | 0 |

CI = Confidence Interval; PCR = Polymerase Chain Reaction; STI = Sexually Transmitted Infection; RTI = Reproductive Tract Infection; HIV = Human immunodeficiency virus

[1] Excludes women who have not been previously pregnant

[2] Therapy against malaria infection (apart from IPTp) after enrolment and before delivery

[3] Anaemia was defined as haemoglobin level < 11grams/decilitre

[4] Confounding is not reflected in *P*-values

[5] Confounding is assessed by observing the difference between the crude odds ratio and adjusted odds ratio. When there is no difference (adjusted / crude – 1) between these two estimates, the observed exposure–outcome effect is not confounded by the potential confounding variable. Variables with odds ratios that changed by 10% or more were considered *a priori* to be potential confounders and we retained for the multivariable model. In this table, only the variable. In this table, no variables demonstrated evidence of confounding on the outcome effect 'any adverse birth outcome'.

[6] Missing values were excluded from the crude odds ratio

[*] Insufficient events to perform stratified analysis for interaction

## Table 3.2 Potential confounding factors for stillbirth

| Potential confounder | Crude analysis | | | Adjusted analysis | | | % change in crude odds ratio[5] | *P*-value for homogeneity | Missing values[6] |
|---|---|---|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | *P*-value[4] | Odds ratio | 95% CI | *P*-value[4] | | | |
| Hypertension at enrolment or delivery | 0.88 | (0.18, 4.21) | 0.875 | 1.00 | (0.19, 5.32) | 1.000 | 13.39 | 0.422 | 86 |
| Labour type | 0.43 | (0.13, 1.46) | 0.163 | 0.39 | (0.11, 1.35) | 0.122 | 9.96 | * | 15 |
| Treatment of malaria infection during pregnancy[2] | 0.56 | (0.15, 2.15) | 0.392 | 0.51 | (0.13, 2.02) | 0.328 | 9.31 | 0.333 | 3 |
| Indoor residual spraying in preceding 12 months | 0.43 | (0.13, 1.46) | 0.162 | 0.39 | (0.11, 1.36) | 0.128 | 8.22 | 0.584 | 26 |
| Gravidae | 0.42 | (0.12, 1.42) | 0.149 | 0.39 | (0.11, 1.34) | 0.121 | 7.42 | 0.573 | 0 |
| Delivery location | 0.42 | (0.12, 1.42) | 0.149 | 0.39 | (0.11, 1.39) | 0.132 | 6.08 | 0.459 | 0 |
| HIV status | 0.42 | (0.12, 1.42) | 0.149 | 0.44 | (0.13, 1.49) | 0.175 | 4.98 | 0.794 | 0 |
| Wealth quintiles | 0.42 | (0.12, 1.42) | 0.149 | 0.44 | (0.14, 1.37) | 0.144 | 4.92 | 0.043 | 0 |
| Delivery type | 0.42 | (0.12, 1.42) | 0.149 | 0.44 | (0.13, 1.48) | 0.173 | 4.55 | * | 0 |
| Maternal haemoglobin level at delivery[3] | 0.57 | (0.15, 2.17) | 0.401 | 0.54 | (0.14, 2.11) | 0.369 | 4.29 | 0.694 | 32 |
| Prior stillbirth[1] | 0.30 | (0.07, 1.39) | 0.103 | 0.32 | (0.07, 1.45) | 0.117 | 4.20 | * | 192 |
| Age of sexual debut (years) | 0.42 | (0.12, 1.42) | 0.149 | 0.44 | (0.13, 1.44) | 0.161 | 4.15 | 0.178 | 0 |
| Placental malaria (PCR diagnosis) | 0.42 | (0.13, 1.44) | 0.155 | 0.44 | (0.13, 1.48) | 0.171 | 3.20 | 0.737 | 7 |
| Type of personnel attending birth | 0.42 | (0.12, 1.42) | 0.149 | 0.41 | (0.12, 1.42) | 0.145 | 3.09 | 0.433 | 0 |
| Bed net ownership | 0.42 | (0.12, 1.42) | 0.149 | 0.43 | (0.13, 1.45) | 0.161 | 2.96 | 0.324 | 0 |
| *N. gonorrhoeae* co-infection (malaria and/or STI/RTI) | 0.42 | (0.12, 1.42) | 0.149 | 0.43 | (0.13, 1.46) | 0.164 | 2.89 | * | 0 |
| Co-infection (malaria and/or STI/RTI) | 0.42 | (0.12, 1.42) | 0.149 | 0.43 | (0.14, 1.36) | 0.139 | 2.60 | 0.045 | 0 |
| Recruitment site | 0.42 | (0.12, 1.42) | 0.149 | 0.43 | (0.13, 1.48) | 0.168 | 2.59 | 0.330 | 0 |
| *C. trachomatis* co-infection (malaria or STI/RTI) | 0.42 | (0.12, 1.42) | 0.149 | 0.41 | (0.12, 1.39) | 0.139 | 2.19 | * | 0 |
| Sex of baby | 0.42 | (0.12, 1.42) | 0.149 | 0.43 | (0.12, 1.48) | 0.168 | 1.89 | 0.673 | 0 |
| Number of lifetime sexual partners | 0.42 | (0.13, 1.43) | 0.154 | 0.43 | (0.13, 1.45) | 0.161 | 1.81 | 0.461 | 6 |

## Table 3.2 Potential confounding factors for stillbirth (continued)

| Potential confounder | Crude analysis | | | Adjusted analysis | | | % change in crude odds ratio[5] | *P*-value for homogeneity | Missing values[6] |
|---|---|---|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | *P*-value[4] | Odds ratio | 95% CI | *P*-value[4] | | | |
| STI/RTI co-infection | 0.42 | (0.13, 1.43) | 0.153 | 0.43 | (0.13, 1.43) | 0.155 | 1.53 | 0.101 | 5 |
| Treatment of STIs/RTIs during pregnancy including syphilis | 0.55 | (0.10, 2.88) | 0.470 | 0.55 | (0.11, 2.93) | 0.481 | 1.35 | * | 258 |
| Syphilis at enrolment (high titre) | 0.42 | (0.13, 1.43) | 0.153 | 0.42 | (0.13, 1.33) | 0.128 | 1.14 | 0.007 | 5 |
| Maternal age at enrolment (years) | 0.42 | (0.12, 1.42) | 0.149 | 0.42 | (0.12, 1.45) | 0.158 | 0.78 | 0.433 | 0 |
| Prior miscarriage[1] | 0.30 | (0.07, 1.39) | 0.103 | 0.31 | (0.07, 1.39) | 0.104 | 0.60 | 0.337 | 192 |
| Prior preterm birth[1] | 0.30 | (0.07, 1.39) | 0.103 | 0.31 | (0.07, 1.40) | 0.105 | 0.52 | * | 192 |
| Bed net usage (on night prior to survey) | 0.42 | (0.12, 1.43) | 0.152 | 0.42 | (0.12, 1.42) | 0.151 | 0.34 | 0.408 | 3 |
| Marital status | 0.42 | (0.12, 1.42) | 0.149 | 0.42 | (0.12, 1.44) | 0.155 | 0.19 | 0.354 | 0 |
| Bacterial vaginosis and STI co-infection | 0.42 | (0.13, 1.43) | 0.153 | 0.42 | (0.13, 1.43) | 0.153 | 0.12 | 0.372 | 5 |
| Treatment of STIs/RTIs during pregnancy excluding syphilis | 0.43 | (0.13, 1.45) | 0.161 | 0.43 | (0.13, 1.45) | 0.162 | 0.07 | * | 13 |
| *T. vaginalis* co-infection (malaria and/or STI/RTI) | 0.42 | (0.12, 1.42) | 0.149 | 0.42 | (0.12, 1.42) | 0.149 | 0.00 | 0.998 | 0 |

CI = Confidence Interval; PCR = Polymerase Chain Reaction; STI = Sexually Transmitted Infection; RTI = Reproductive Tract Infection; HIV = Human immunodeficiency virus

[1] Excludes women who have not been previously pregnant

[2] Therapy against malaria infection (apart from IPTp) after enrolment and before delivery

[3] Anaemia was defined as haemoglobin level < 11grams/decilitre

[4] Confounding is not reflected in *P*-values

[5] Confounding is assessed by observing the difference between the crude odds ratio and adjusted odds ratio. When there is no difference (adjusted / crude – 1) between these two estimates, the observed exposure–outcome effect is not confounded by the potential confounding variable. Variables with odds ratios that changed by 10% or more were considered *a priori* to be potential confounders and we retained for the multivariable model. In this table, only the variable. 'Hypertension at enrolment or delivery' demonstrated evidence of confounding on the outcome effect 'stillbirth'.

[6] Missing values were excluded from the crude odds ratio

[*] Insufficient events to perform stratified analysis for interaction

## Table 3.3 Potential confounding factors for low birth weight

| Potential confounder | Crude analysis | | | Adjusted analysis | | | % change in crude odds ratio[5] | *P*-value for homogeneity | Missing values[6] |
|---|---|---|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | *P*-value[4] | Odds ratio | 95% CI | *P*-value[4] | | | |
| Gravidae | 0.78 | (0.49, 1.23) | 0.279 | 0.69 | (0.42, 1.11) | 0.122 | 11.86 | 0.952 | 0 |
| Prior miscarriage[1] | 0.68 | (0.21, 2.19) | 0.513 | 0.64 | (0.19, 2.14) | 0.461 | 6.17 | 0.623 | 620 |
| Treatment of malaria infection during pregnancy[2] | 0.78 | (0.50, 1.23) | 0.288 | 0.74 | (0.47, 1.18) | 0.207 | 4.87 | 0.034 | 3 |
| Co-infection (malaria and/or STI/RTI) | 0.78 | (0.49, 1.23) | 0.279 | 0.74 | (0.47, 1.17) | 0.202 | 4.43 | 0.211 | 0 |
| Marital status | 0.78 | (0.49, 1.23) | 0.279 | 0.75 | (0.48, 1.18) | 0.210 | 3.72 | 0.079 | 0 |
| Placental malaria (PCR diagnosis) | 0.79 | (0.50, 1.25) | 0.310 | 0.77 | (0.49, 1.21) | 0.255 | 2.98 | 0.964 | 7 |
| Indoor residual spraying in preceding 12 months | 0.73 | (0.46, 1.15) | 0.175 | 0.71 | (0.45, 1.13) | 0.142 | 2.78 | 0.450 | 26 |
| HIV status | 0.78 | (0.49, 1.23) | 0.279 | 0.80 | (0.51, 1.26) | 0.331 | 2.51 | 0.622 | 0 |
| Sex of baby | 0.78 | (0.49, 1.23) | 0.279 | 0.80 | (0.51, 1.25) | 0.322 | 2.16 | 0.364 | 0 |
| Delivery location | 0.78 | (0.49, 1.23) | 0.279 | 0.79 | (0.50, 1.25) | 0.321 | 1.90 | 0.021 | 0 |
| Bed net ownership | 0.78 | (0.49, 1.23) | 0.279 | 0.79 | (0.50, 1.26) | 0.324 | 1.88 | 0.354 | 0 |
| Wealth quintiles | 0.78 | (0.49, 1.23) | 0.279 | 0.77 | (0.48, 1.21) | 0.254 | 1.71 | 0.980 | 0 |
| Maternal age at enrolment (years) | 0.78 | (0.49, 1.23) | 0.279 | 0.77 | (0.48, 1.23) | 0.271 | 1.65 | 0.159 | 0 |
| Number of lifetime sexual partners | 0.77 | (0.49, 1.22) | 0.265 | 0.78 | (0.50, 1.24) | 0.295 | 1.58 | 0.010 | 6 |
| Syphilis at enrolment (high titre) | 0.78 | (0.49, 1.23) | 0.281 | 0.77 | (0.49, 1.21) | 0.253 | 1.54 | * | 5 |
| Delivery type | 0.78 | (0.49, 1.23) | 0.279 | 0.79 | (0.50, 1.24) | 0.305 | 1.30 | 0.546 | 0 |
| Treatment of STIs/RTIs during pregnancy including syphilis | 1.09 | (0.60, 1.96) | 0.777 | 1.10 | (0.61, 1.99) | 0.752 | 1.07 | 0.932 | 258 |
| Bed net usage (on night prior to survey) | 0.77 | (0.49, 1.21) | 0.253 | 0.76 | (0.48, 1.21) | 0.242 | 1.06 | 0.203 | 3 |
| Age of sexual debut (years) | 0.78 | (0.49, 1.23) | 0.279 | 0.78 | (0.50, 1.24) | 0.295 | 0.81 | 0.775 | 0 |
| Labour type | 0.78 | (0.49, 1.23) | 0.283 | 0.77 | (0.49, 1.22) | 0.271 | 0.70 | * | 15 |
| *N.gonorrhoeae* co-infection (malaria and/or STI/RTI) | 0.78 | (0.49, 1.23) | 0.279 | 0.78 | (0.50, 1.23) | 0.290 | 0.49 | 0.592 | 0 |

## Table 3.3 Potential confounding factors for low birth weight (continued)

| Potential confounder | Crude analysis | | | Adjusted analysis | | | % change in crude odds ratio[5] | *P*-value for homogeneity | Missing values[6] |
|---|---|---|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | *P*-value[4] | Odds ratio | 95% CI | *P*-value[4] | | | |
| STI/RTI co-infection | 0.78 | (0.49, 1.23) | 0.281 | 0.78 | (0.49, 1.22) | 0.273 | 0.40 | 0.693 | 5 |
| *T. vaginalis* co-infection (malaria and/or STI/RTI) | 0.78 | (0.49, 1.23) | 0.279 | 0.78 | (0.50, 1.22) | 0.279 | 0.31 | 0.035 | 0 |
| Prior preterm birth[1] | 0.72 | (0.40, 1.29) | 0.267 | 0.72 | (0.41, 1.28) | 0.266 | 0.29 | 0.019 | 192 |
| Maternal haemoglobin level at delivery[3] | 0.80 | (0.50, 1.28) | 0.354 | 0.80 | (0.50, 1.28) | 0.349 | 0.21 | 0.120 | 32 |
| Bacterial vaginosis and STI co-infection | 0.78 | (0.49, 1.23) | 0.281 | 0.78 | (0.50, 1.22) | 0.272 | 0.21 | 0.060 | 5 |
| *C. trachomatis* co-infection (malaria or STI/RTI) | 0.78 | (0.49, 1.23) | 0.279 | 0.78 | (0.49, 1.23) | 0.284 | 0.17 | 0.435 | 0 |
| Hypertension at enrolment or delivery | 0.76 | (0.48, 1.23) | 0.264 | 0.76 | (0.48, 1.23) | 0.267 | 0.13 | 0.847 | 86 |
| Type of personnel attending birth | 0.78 | (0.49, 1.23) | 0.279 | 0.78 | (0.50, 1.23) | 0.279 | 0.10 | 0.004 | 0 |
| Treatment of STIs/RTIs during pregnancy excluding syphilis | 0.78 | (0.49, 1.23) | 0.279 | 0.78 | (0.49, 1.23) | 0.283 | 0.08 | 0.826 | 0 |
| Recruitment site | 0.78 | (0.49, 1.23) | 0.279 | 0.78 | (0.50, 1.22) | 0.277 | 0.07 | 0.053 | 0 |
| Prior stillbirth[1] | 0.68 | (0.21, 2.19) | 0.513 | 0.68 | (0.20, 2.31) | 0.531 | 0.02 | 0.876 | 620 |

CI = Confidence Interval; PCR = Polymerase Chain Reaction; STI = Sexually Transmitted Infection; RTI = Reproductive Tract Infection; HIV = Human immunodeficiency virus

[1] Excludes women who have not been previously pregnant

[2] Therapy against malaria infection (apart from IPTp) after enrolment and before delivery

[3] Anaemia was defined as haemoglobin level < 11grams/decilitre

[4] Confounding is not reflected in *P*-values

[5] Confounding is assessed by observing the difference between the crude odds ratio and adjusted odds ratio. When there is no difference (adjusted / crude – 1) between these two estimates, the observed exposure–outcome effect is not confounded by the potential confounding variable. Variables with odds ratios that changed by 10% or more were considered *a priori* to be potential confounders and we retained for the multivariable model. In this table, only the variable. 'Hypertension at enrolment or delivery' demonstrated evidence of confounding on the outcome effect 'stillbirth'.

[6] Missing values were excluded from the crude odds ratio

[*] Insufficient events to perform stratified analysis for interaction

## Table 3.4 Potential confounding factors for preterm delivery

| Potential confounder | Crude analysis | | | Adjusted analysis | | | % change in crude odds ratio[5] | *P*-value for homogeneity | Missing values[6] |
|---|---|---|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | *P*-value[4] | Odds ratio | 95% CI | *P*-value[4] | | | |
| Prior preterm birth[1] | 0.35 | (0.11, 1.08) | 0.056 | 0.30 | (0.09, 0.96) | 0.031 | 14.04 | * | 620 |
| Prior miscarriage[1] | 0.35 | (0.11, 1.08) | 0.056 | 0.32 | (0.10, 1.01) | 0.040 | 8.67 | 0.326 | 620 |
| Gravidae | 0.36 | (0.23, 0.55) | 0.000 | 0.34 | (0.22, 0.52) | 0.000 | 5.63 | 0.794 | 0 |
| Sex of baby | 0.36 | (0.23, 0.55) | 0.000 | 0.37 | (0.24, 0.57) | 0.000 | 4.25 | 0.237 | 0 |
| Maternal age at enrolment (years) | 0.36 | (0.23, 0.55) | 0.000 | 0.34 | (0.22, 0.53) | 0.000 | 3.67 | 0.285 | 0 |
| Prior stillbirth[1] | 0.31 | (0.19, 0.51) | 0.000 | 0.30 | (0.18, 0.50) | 0.000 | 3.29 | 0.032 | 192 |
| Labour type | 0.36 | (0.24, 0.56) | 0.000 | 0.35 | (0.23, 0.55) | 0.000 | 2.43 | * | 15 |
| Delivery type | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.24, 0.56) | 0.000 | 2.29 | 0.336 | 0 |
| Syphilis at enrolment (high titre) | 0.36 | (0.23, 0.55) | 0.000 | 0.35 | (0.23, 0.54) | 0.000 | 2.23 | * | 5 |
| Hypertension at enrolment or delivery | 0.35 | (0.22, 0.54) | 0.000 | 0.36 | (0.23, 0.55) | 0.000 | 2.22 | 0.021 | 86 |
| Co-infection (malaria and/or STI/RTI) | 0.36 | (0.23, 0.55) | 0.000 | 0.35 | (0.23, 0.54) | 0.000 | 2.08 | 0.248 | 0 |
| Type of personnel attending birth | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.24, 0.56) | 0.000 | 1.87 | 0.128 | 0 |
| Number of lifetime sexual partners | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.24, 0.56) | 0.000 | 1.57 | 0.297 | 6 |
| *N. gonorrhoeae* co-infection (malaria and/or STI/RTI) | 0.36 | (0.23, 0.55) | 0.000 | 0.35 | (0.23, 0.54) | 0.000 | 1.56 | 0.331 | 0 |
| Age of sexual debut (years) | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.24, 0.55) | 0.000 | 1.37 | 0.734 | 0 |
| Maternal haemoglobin level at delivery[3] | 0.37 | (0.24, 0.57) | 0.000 | 0.37 | (0.24, 0.57) | 0.000 | 1.35 | 0.212 | 32 |
| Marital status | 0.36 | (0.23, 0.55) | 0.000 | 0.35 | (0.23, 0.54) | 0.000 | 1.29 | 0.038 | 0 |
| Placental malaria (PCR diagnosis) | 0.36 | (0.24, 0.55) | 0.000 | 0.36 | (0.23, 0.55) | 0.000 | 1.10 | 0.955 | 7 |
| Bed net ownership | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.23, 0.55) | 0.000 | 1.07 | 0.659 | 0 |
| STI/RTI co-infection | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.23, 0.55) | 0.000 | 0.91 | 0.984 | 5 |
| *T. vaginalis* co-infection (malaria and/or STI/RTI) | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.23, 0.55) | 0.000 | 0.89 | 0.053 | 0 |

## Table 3.4 Potential confounding factors for preterm delivery (continued)

| Potential confounder | Crude analysis | | | Adjusted analysis | | | % change in crude odds ratio[5] | *P*-value for homogeneity | Missing values[6] |
|---|---|---|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | *P*-value[4] | Odds ratio | 95% CI | *P*-value[4] | | | |
| HIV status | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.23, 0.55) | 0.000 | 0.84 | 0.805 | 0 |
| Wealth quintiles | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.23, 0.55) | 0.000 | 0.70 | 0.461 | 0 |
| Recruitment site | 0.36 | (0.23, 0.55) | 0.000 | 0.35 | (0.23, 0.54) | 0.000 | 0.66 | 0.316 | 0 |
| Indoor residual spraying in preceding 12 months | 0.32 | (0.21, 0.50) | 0.000 | 0.33 | (0.21, 0.50) | 0.000 | 0.57 | 0.450 | 26 |
| Bed net usage (on night prior to survey) | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.23, 0.55) | 0.000 | 0.57 | 0.894 | 3 |
| Delivery location | 0.36 | (0.23, 0.55) | 0.000 | 0.35 | (0.23, 0.55) | 0.000 | 0.49 | 0.089 | 0 |
| Treatment of STIs/RTIs during pregnancy excluding syphilis | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.23, 0.55) | 0.000 | 0.18 | 0.623 | 0 |
| Bacterial vaginosis and STI co-infection | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.23, 0.55) | 0.000 | 0.18 | 0.168 | 5 |
| *C. trachomatis* co-infection (malaria or STI/RTI) | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.23, 0.55) | 0.000 | 0.09 | 0.903 | 0 |
| Treatment of STIs/RTIs during pregnancy including syphilis | 0.46 | (0.27, 0.77) | 0.003 | 0.46 | (0.27, 0.77) | 0.003 | 0.09 | 0.384 | 258 |
| Treatment of malaria infection during pregnancy[2] | 0.36 | (0.23, 0.55) | 0.000 | 0.36 | (0.23, 0.55) | 0.000 | 0.05 | 0.172 | 3 |

CI = Confidence Interval; PCR = Polymerase Chain Reaction; STI = Sexually Transmitted Infection; RTI = Reproductive Tract Infection; HIV = Human immunodeficiency virus

[1] Excludes women who have not been previously pregnant

[2] Therapy against malaria infection (apart from IPTp) after enrolment and before delivery

[3] Anaemia was defined as haemoglobin level < 11grams/decilitre

[4] Confounding is not reflected in *P*-values

[5] Confounding is assessed by observing the difference between the crude odds ratio and adjusted odds ratio. When there is no difference (adjusted / crude – 1) between these two estimates, the observed exposure–outcome effect is not confounded by the potential confounding variable. Variables with odds ratios that changed by 10% or more were considered *a priori* to be potential confounders and we retained for the multivariable model. In this table, only the variable 'Hypertension at enrolment or delivery' demonstrated evidence of confounding on the outcome effect 'stillbirth'.

[6] Missing values were excluded from the crude odds ratio

[*] Insufficient events to perform stratified analysis for interaction

## Table 3.5 Potential confounding factors for intrauterine growth retardation

| Potential confounder | Crude analysis | | | Adjusted analysis | | | % change in crude odds ratio[5] | *P*-value for homogeneity | Missing values[6] |
|---|---|---|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | *P*-value[4] | Odds ratio | 95% CI | *P*-value[4] | | | |
| Prior miscarriage[1] | 0.71 | (0.13, 3.92) | 0.697 | 0.90 | (0.13, 6.01) | 0.912 | 25.73 | 0.938 | 620 |
| Hypertension at enrolment or delivery | 1.55 | (0.68, 3.56) | 0.295 | 1.68 | (0.72, 3.94) | 0.228 | 8.25 | 0.883 | 86 |
| Gravidae | 1.85 | (0.82, 4.19) | 0.134 | 1.70 | (0.75, 3.87) | 0.198 | 7.89 | 0.458 | 0 |
| Maternal age at enrolment (years) | 1.85 | (0.82, 4.19) | 0.134 | 1.72 | (0.75, 3.90) | 0.192 | 7.28 | 0.419 | 0 |
| Number of lifetime sexual partners | 1.82 | (0.80, 4.12) | 0.148 | 1.91 | (0.83, 4.43) | 0.124 | 5.34 | 0.332 | 6 |
| Treatment of STIs/RTIs during pregnancy including syphilis | 3.19 | (0.95, 10.74) | 0.048 | 3.32 | (0.98, 11.29) | 0.042 | 4.11 | 0.724 | 258 |
| Placental malaria (PCR diagnosis) | 1.88 | (0.83, 4.27) | 0.123 | 1.81 | (0.79, 4.11) | 0.153 | 4.10 | 0.981 | 7 |
| Co-infection (malaria and/or STI/RTI) | 1.85 | (0.82, 4.19) | 0.134 | 1.78 | (0.79, 4.02) | 0.161 | 3.93 | 0.348 | 0 |
| Prior stillbirth[1] | 0.71 | (0.13, 3.92) | 0.697 | 0.74 | (0.13, 4.07) | 0.729 | 3.70 | * | 620 |
| Treatment of malaria infection during pregnancy[2] | 1.86 | (0.82, 4.21) | 0.131 | 1.79 | (0.79, 4.07) | 0.157 | 3.62 | 0.459 | 3 |
| Bed net ownership | 1.85 | (0.82, 4.19) | 0.134 | 1.90 | (0.83, 4.33) | 0.122 | 2.51 | 0.609 | 0 |
| Indoor residual spraying in preceding 12 months | 1.83 | (0.81, 4.16) | 0.142 | 1.79 | (0.79, 4.06) | 0.161 | 2.50 | 0.710 | 26 |
| Sex of baby | 1.85 | (0.82, 4.19) | 0.134 | 1.89 | (0.84, 4.28) | 0.120 | 2.24 | 0.582 | 0 |
| Wealth quintiles | 1.85 | (0.82, 4.19) | 0.134 | 1.81 | (0.79, 4.13) | 0.151 | 2.05 | 0.950 | 0 |
| Marital status | 1.85 | (0.82, 4.19) | 0.134 | 1.81 | (0.83, 3.99) | 0.132 | 1.95 | 0.037 | 0 |
| Prior preterm birth[1] | 0.71 | (0.13, 3.92) | 0.697 | 0.73 | (0.13, 3.99) | 0.713 | 1.82 | * | 620 |
| *T. vaginalis* co-infection (malaria and/or STI/RTI) | 1.85 | (0.82, 4.19) | 0.134 | 1.88 | (0.84, 4.21) | 0.117 | 1.74 | 0.171 | 0 |
| Treatment of STIs/RTIs during pregnancy excluding syphilis | 1.91 | (0.84, 4.33) | 0.116 | 1.94 | (0.85, 4.41) | 0.108 | 1.62 | 0.685 | 13 |
| Delivery location | 1.85 | (0.82, 4.19) | 0.134 | 1.88 | (0.83, 4.26) | 0.127 | 1.34 | * | 0 |
| Maternal haemoglobin level at delivery[3] | 1.86 | (0.82, 4.23) | 0.130 | 1.89 | (0.83, 4.27) | 0.121 | 1.27 | 0.469 | 32 |
| Labour type | 1.86 | (0.82, 4.21) | 0.132 | 1.88 | (0.83, 4.27) | 0.125 | 1.27 | * | 15 |

## Table 5.5 Potential confounding factors for intrauterine growth retardation (continued)

| Potential confounder | Crude analysis | | | Adjusted analysis | | | % change in crude odds ratio[5] | *P*-value for homogeneity | Missing values[6] |
|---|---|---|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | *P*-value[4] | Odds ratio | 95% CI | *P*-value[4] | | | |
| HIV status | 1.85 | (0.82, 4.19) | 0.134 | 1.87 | (0.82, 4.23) | 0.128 | 0.98 | 0.757 | 0 |
| *N. gonorrhoeae* co-infection (malaria and/or STI/RTI) | 1.85 | (0.82, 4.19) | 0.134 | 1.87 | (0.82, 4.24) | 0.129 | 0.91 | * | 0 |
| Bed net usage (on night prior to survey) | 1.81 | (0.80, 4.10) | 0.151 | 1.80 | (0.79, 4.11) | 0.159 | 0.59 | 0.328 | 3 |
| Recruitment site | 1.85 | (0.82, 4.19) | 0.134 | 1.84 | (0.82, 4.14) | 0.134 | 0.51 | 0.058 | 0 |
| STI/RTI co-infection | 1.87 | (0.82, 4.24) | 0.128 | 1.86 | (0.82, 4.22) | 0.129 | 0.31 | 0.407 | 5 |
| *C. trachomatis* co-infection (malaria or STI/RTI) | 1.85 | (0.82, 4.19) | 0.134 | 1.86 | (0.82, 4.22) | 0.134 | 0.26 | 0.398 | 0 |
| Type of personnel attending birth | 1.85 | (0.82, 4.19) | 0.134 | 1.85 | (0.82, 4.17) | 0.134 | 0.24 | 0.057 | 0 |
| Age of sexual debut (years) | 1.85 | (0.82, 4.19) | 0.134 | 1.85 | (0.83, 4.17) | 0.128 | 0.24 | 0.333 | 0 |
| Bacterial vaginosis and STI co-infection | 1.87 | (0.82, 4.24) | 0.128 | 1.87 | (0.84, 4.19) | 0.121 | 0.16 | 0.135 | 5 |
| Syphilis at enrolment (high titre) | 1.87 | (0.82, 4.24) | 0.128 | 1.87 | (0.82, 4.24) | 0.129 | 0.10 | * | 5 |
| Delivery type | 1.85 | (0.82, 4.19) | 0.134 | 1.85 | (0.82, 4.16) | 0.131 | 0.03 | 0.087 | 0 |

CI = Confidence Interval; PCR = Polymerase Chain Reaction; STI = Sexually Transmitted Infection; RTI = Reproductive Tract Infection; HIV = Human immunodeficiency virus

[1] Excludes women who have not been previously pregnant

[2] Therapy against malaria infection (apart from IPTp) after enrolment and before delivery

[3] Anaemia was defined as haemoglobin level < 11grams/decilitre

[4] Confounding is not reflected in *P*-values

[5] Confounding is assessed by observing the difference between the crude odds ratio and adjusted odds ratio. When there is no difference (adjusted / crude – 1) between these two estimates, the observed exposure–outcome effect is not confounded by the potential confounding variable. Variables with odds ratios that changed by 10% or more were considered *a priori* to be potential confounders and we retained for the multivariable model. In this table, only the variable. 'Hypertension at enrolment or delivery' demonstrated evidence of confounding on the outcome effect 'stillbirth'.

[6] Missing values were excluded from the crude odds ratio

[*] Insufficient events to perform stratified analysis for interaction

## Results

In this analysis the effects of IPTp-SP on adverse birth outcomes were explored depending on the status of malaria infection and curable STIs/RTIs. Specifically, four adverse birth outcomes were investigated in a dose-response relationship: (i) stillbirth, (ii) LBW, (iii) preterm birth, and (iv) intrauterine growth retardation in seven categories of maternal malaria and STI/RTI categories.

There were no significant differences in the prevalence of malaria infection and curable STIs/RTIs at baseline across IPTp-SP exposure groups stratified by 0 to 1 dose (n=126) versus ≥ 2 doses (n=590) as shown in Table 3.6 (enrolment) and Table 3.7 (delivery) and, separately, 2 doses (n=310) versus ≥ 3 doses (n=280) in Table 3.8 (enrolment) and Table 3.9 (delivery). However, the odds of any adverse birth outcome amongst women who received ≥ 2 doses compared to 0-1, presented in Table 3.10, were reduced 45% (OR 0.55, 95% CI: 0.36, 0.86). The odds of any adverse birth outcome were reduced 12% further (57% total reduction) with ≥ 3 doses (OR 0.43, 95% CI 0.27, 0.68). Two doses, compared to 0 to 1 dose, reduced the odds of preterm delivery by 58% (OR 0.42, 95% CI: 0.27, 0.67). Three or more doses of IPTp reduced the odds of preterm birth 21% further (79% total reduction) (OR 0.21, 95% CI 0.13, 0.35) as shown in Table 3.10. A similar dose-response relationship was also observed against malaria infection and STIs/RTIs in dosing categories of 0-1 versus ≥ 2 doses, and 2 versus ≥ 3 doses.

Across 24 of 30 infection categories, ≥ 2 doses conferred greater protection against adverse birth outcomes than 0-1 dose, of which eight categories showed statistically significant effects as shown in Table 3.11; three categories related to any birth outcome (malaria only, *N. gonorrhoeae* and/or *C. trachomatis*, and no identified

infection), one was specific to LBW (no identified infection), and four were associated with preterm birth (malaria only, malaria and *T. vaginalis* and/or bacterial vaginosis, and no identified infection).

**Table 3.6 Participant characteristics at enrolment: 0-1 dose vs. ≥ 2 doses of IPTp-SP**

| Characteristics at enrolment | Doses of SP received – number (%)* | | | | *P*-value |
|---|---|---|---|---|---|
| | 0-1 dose (n = 126) | | ≥ 2 doses(n = 590) | | |
| **Age of participants** | | | | | 0.498 |
| Mean (SD) | 25.8 | (6.5) | 25.4 | (6.4) | |
| Median (IQR) | 24.0 | (20.0, 31.0) | 24.0 | (20.0, 30.0) | |
| **Marital status** | | | | | 0.175 |
| Single | 19 | (15.1) | 123 | (20.8) | |
| Married, divorced/separated or widowed | 107 | (84.9) | 467 | (79.2) | |
| **Age at sexual debut** | | | | | 0.733 |
| < 15 years of age | 13 | (10.3) | 49 | (8.3) | |
| ≥ 15 years of age | 96 | (76.2) | 455 | (77.1) | |
| Unknown | 17 | (13.5) | 86 | (14.6) | |
| **Number of lifetime sexual partners** | | | | | 0.362 |
| 1 partner | 52 | (41.3) | 272 | (46.6) | |
| 2 partners | 45 | (35.7) | 161 | (27.6) | |
| 3 partners | 18 | (14.3) | 94 | (16.1) | |
| 4 or more partners | 11 | (8.7) | 57 | (9.8) | |
| **Gravidae** | | | | | 0.301 |
| Primigravidae | 27 | (21.4) | 165 | (28.0) | |
| Secundigravidae | 19 | (15.1) | 77 | (13.1) | |
| Multigravidae | 80 | (63.5) | 348 | (59.0) | |
| **Wealth Quintiles** | | | | | 0.048 |
| Lowest | 21 | (16.7) | 115 | (19.5) | |
| Second | 32 | (25.4) | 111 | (18.8) | |
| Middle | 31 | (24.6) | 113 | (19.2) | |
| Fourth | 14 | (11.1) | 122 | (20.7) | |
| Highest | 28 | (22.2) | 129 | (21.9) | |
| **Bed net ownership** | | | | | 0.493 |
| No | 68 | (54.0) | 297 | (50.3) | |
| Yes | 58 | (46.0) | 293 | (49.7) | |
| **Used insecticide treated net on previous night** | | | | | 0.840 |
| No | 77 | (61.1) | 366 | (62.4) | |
| Yes | 49 | (38.9) | 221 | (37.6) | |
| Missing | 0 | | 3 | | |
| **Indoor residual spraying in the previous 12 months** | | | | | 0.186 |
| No | 103 | (83.1) | 439 | (77.6) | |
| Yes | 21 | (16.9) | 127 | (22.4) | |
| Missing | 2 | | 24 | | |

**Table 3.6 Participant characteristics at enrolment: 0-1 dose vs. ≥ 2 doses of IPTp-SP (continued)**

| Characteristics at enrolment | Doses of SP received – number (%)* | | | | *P*-value |
|---|---|---|---|---|---|
| | 0-1 dose (n = 126) | | ≥ 2 doses(n = 590) | | |
| **Experienced miscarriage before** | | | | | 0.869 |
| No | 86 | (86.9) | 371 | (87.3) | |
| Yes | 13 | (13.1) | 54 | (12.7) | |
| None reported by primigravidae | 27 | | 165 | | |
| **Delivered a premature baby before** | | | | | 1.000 |
| No | 94 | (94.9) | 401 | (94.4) | |
| Yes | 5 | (5.1) | 24 | (5.6) | |
| Not applicable to primigravidae | 27 | | 165 | | |
| **Delivered a stillborn before** | | | | | 0.307 |
| No | 94 | (94.9) | 387 | (91.1) | |
| Yes | 5 | (5.1) | 38 | (8.9) | |
| Not applicable to primigravidae | 27 | | 165 | | |
| **HIV status** | | | | | 0.186 |
| Negative | 105 | (83.3) | 519 | (88.0) | |
| Positive | 21 | (16.7) | 71 | (12.0) | |
| **Malaria and curable STIs/RTIs** | | | | | |
| Malaria (PCR diagnosis) | 62 | (49.2) | 346 | (59.3) | 0.047 |
| Syphilis (high titre) | 1 | (0.8) | 17 | (2.9) | 0.223 |
| *Neisseria gonorrhoeae* | 1 | (0.8) | 21 | (3.6) | 0.152 |
| *Chlamydia trachomatis* | 8 | (6.3) | 26 | (4.4) | 0.357 |
| *Trichomonas vaginalis* | 30 | (23.8) | 140 | (23.7) | 1.000 |
| Bacterial vaginosis | 59 | (46.8) | 277 | (46.9) | 1.000 |

* Age is shown as the median value with the interquartile range in parentheses. *P*-values are from Wilcoxon rank sum test (continuous variables) or Fisher's exact test (categorical variables). PCR = polymerase chain reaction

**Table 3.7 Participant characteristics at delivery: 0-1 dose vs. ≥ 2 doses of IPTp-SP**

| Characteristics at delivery | Doses of SP received – number (%)* | | | | *P*-value |
|---|---|---|---|---|---|
| | 0 - 1 dose (n = 126) | | ≥ 2 doses (n = 590) | | |
| **Place of delivery** | | | | | 0.233 |
| Hospital | 119 | (94.4) | 551 | (93.4) | |
| Clinic | 1 | (0.8) | 19 | (3.2) | |
| Home | 6 | (4.8) | 20 | (3.4) | |
| **Delivery performed by** | | | | | 0.240 |
| Doctor | 3 | (2.4) | 38 | (6.4) | |
| Midwife | 115 | (91.3) | 524 | (88.8) | |
| Family member | 5 | (4.0) | 17 | (2.9) | |
| Other | 3 | (2.4) | 11 | (1.9) | |
| **Type of labour** | | | | | 0.296 |
| Spontaneous | 126 | (100.0) | 558 | (97.4) | |
| Induced | 0 | (0.0) | 9 | (1.6) | |
| Augmented | 0 | (0.0) | 6 | (1.0) | |
| **Type of delivery** | | | | | 0.092 |
| Vaginal | 123 | (97.6) | 551 | (93.4) | |
| C-section | 3 | (2.4) | 39 | (6.6) | |
| **Hypertension** | | | | | 0.296 |
| No | 110 | (96.5) | 506 | (98.1) | |
| Yes | 4 | (3.5) | 10 | (1.9) | |
| **Maternal haemoglobin** | | | | | 0.786 |
| Normal | 103 | (85.1) | 470 | (83.5) | |
| Anaemic | 18 | (14.9) | 93 | (16.5) | |
| **Sex of baby** | | | | | 0.008 |
| Female | 78 | (61.9) | 287 | (48.6) | |
| Male | 48 | (38.1) | 303 | (51.4) | |
| **Received curative treatment for malaria infection** | | | | | 0.102 |
| No | 115 | (92.0) | 508 | (86.4) | |
| Yes | 10 | (8.0) | 80 | (13.6) | |
| **Received curative treatment for any STI/RTI** | | | | | 1.000 |
| Untreated | 116 | (92.1) | 540 | (91.5) | |
| Treated | 10 | (7.9) | 50 | (8.5) | |

* Age is shown as the median value with the interquartile range in parentheses. *P*-values are from Wilcoxon rank sum test (continuous variables) or Fisher's exact test (categorical variables). PCR = polymerase chain reaction

**Table 3.8 Participant characteristics at enrolment: 2 doses vs. ≥ 3 doses of IPTp-SP**

| Characteristics at enrolment | Doses of SP received – number (%) | | | | *P*-value |
|---|---|---|---|---|---|
| | 2 doses (n = 310) | | ≥ 3 doses (n = 280) | | |
| **Age of participants** | | | | | 0.521 |
| Mean (SD)* | 25.6 | (6.4) | 25.2 | (6.4) | |
| Median (IQR)* | 25.0 | (20.0, 30.0) | 24.0 | (20.0, 30.0) | |
| **Marital status** | | | | | 0.479 |
| Single | 61 | (19.7) | 62 | (22.1) | |
| Married, divorced/separated or widowed | 249 | (80.3) | 218 | (77.9) | |
| **Age at sexual debut** | | | | | 0.328 |
| < 15 years of age | 21 | (6.8) | 28 | (10.0) | |
| ≥ 15 years of age | 241 | (77.7) | 214 | (76.4) | |
| Unknown | 48 | (15.5) | 38 | (13.6) | |
| **Number of lifetime sexual partners** | | | | | 0.460 |
| 1 partner | 150 | (49.0) | 122 | (43.9) | |
| 2 partners | 83 | (27.1) | 78 | (28.1) | |
| 3 partners | 48 | (15.7) | 46 | (16.5) | |
| 4 or more partners | 25 | (8.2) | 32 | (11.5) | |
| **Gravidae** | | | | | 0.936 |
| Primigravidae | 86 | (27.7) | 79 | (28.2) | |
| Secundigravidae | 42 | (13.5) | 35 | (12.5) | |
| Multigravidae | 182 | (58.7) | 166 | (59.3) | |
| **Wealth Quintiles** | | | | | 0.379 |
| Lowest | 68 | (21.9) | 47 | (16.8) | |
| Second | 58 | (18.7) | 53 | (18.9) | |
| Middle | 59 | (19.0) | 54 | (19.3) | |
| Fourth | 56 | (18.1) | 66 | (23.6) | |
| Highest | 69 | (22.3) | 60 | (21.4) | |
| **Bed net ownership** | | | | | 0.564 |
| No | 160 | (51.6) | 137 | (48.9) | |
| Yes | 150 | (48.4) | 143 | (51.1) | |
| **Used insecticide treated net on previous night** | | | | | 0.932 |
| No | 193 | (62.7) | 173 | (62.0) | |
| Yes | 115 | (37.3) | 106 | (38.0) | |
| Missing | 2 | | 1 | | |
| **Indoor residual spraying in the previous 12 months** | | | | | 0.002 |
| No | 247 | (82.9) | 192 | (71.6) | |
| Yes | 51 | (17.1) | 76 | (28.4) | |
| Missing | 12 | | 12 | | |

**Table 3.8 Participant characteristics at enrolment: 2 doses vs. ≥ 3 doses of IPTp-SP (continued)**

| Characteristics at enrolment | Doses of SP received – number (%) | | | | *P*-value |
|---|---|---|---|---|---|
| | 2 doses (n = 310) | | ≥ 3 doses (n = 280) | | |
| **Experienced miscarriage before** | | | | | 0.470 |
| No | 193 | (86.2) | 178 | (88.6) | |
| Yes | 31 | (13.8) | 23 | (11.4) | |
| None reported by primigravidae | 86 | | 79 | | |
| **Delivered a premature baby before** | | | | | 0.297 |
| No | 214 | (95.5) | 187 | (93.0) | |
| Yes | 10 | (4.5) | 14 | (7.0) | |
| Not applicable to primigravidae | 86 | | 79 | | |
| **Delivered a stillborn before** | | | | | 0.737 |
| No | 205 | (91.5) | 182 | (90.5) | |
| Yes | 19 | (8.5) | 19 | (9.5) | |
| Not applicable to primigravidae | 86 | | 79 | | |
| **HIV status** | | | | | 0.528 |
| Negative | 270 | (87.1) | 249 | (88.9) | |
| Positive | 40 | (12.9) | 31 | (11.1) | |
| **Malaria and curable STIs/RTIs** | | | | | |
| Malaria (PCR diagnosis) | 171 | (56.1) | 175 | (62.9) | 0.092 |
| Syphilis (high titre) | 7 | (2.3) | 10 | (3.6) | 0.462 |
| *Neisseria gonorrhoeae* | 15 | (4.8) | 6 | (2.1) | 0.117 |
| *Chlamydia trachomatis* | 16 | (5.2) | 10 | (3.6) | 0.423 |
| *Trichomonas vaginalis* | 73 | (23.5) | 67 | (23.9) | 0.923 |
| Bacterial vaginosis | 145 | (46.8) | 132 | (47.1) | 0.934 |

* Age is shown as the median value with the interquartile range in parentheses. *P*-values are from Wilcoxon rank sum test (continuous variables) or Fisher's exact test (categorical variables). PCR = polymerase chain reaction

**Table 3.9 Participant characteristics at delivery: 2 doses vs. ≥ 3 doses of IPTp-SP**

| Characteristics at enrolment | Doses of SP received – number (%) | | | | *P*-value |
|---|---|---|---|---|---|
| | 2 doses (n = 310) | | ≥ 3 doses (n = 280) | | |
| **Place of delivery** | | | | | 0.418 |
| Hospital | 292 | (94.2) | 259 | (92.5) | |
| Clinic | 7 | (2.3) | 12 | (4.3) | |
| Home | 11 | (3.5) | 9 | (3.2) | |
| **Delivery performed by** | | | | | 0.782 |
| Doctor | 20 | (6.5) | 18 | (6.4) | |
| Midwife | 277 | (89.4) | 247 | (88.2) | |
| Family member | 9 | (2.9) | 8 | (2.9) | |
| Other | 4 | (1.3) | 7 | (2.5) | |
| **Type of labour** | | | | | 0.920 |
| Spontaneous | 292 | (97.7) | 266 | (97.1) | |
| Induced | 4 | (1.3) | 5 | (1.8) | |
| Augmented | 3 | (1.0) | 3 | (1.1) | |
| **Type of delivery** | | | | | 0.870 |
| Vaginal | 290 | (93.5) | 261 | (93.2) | |
| C-section | 20 | (6.5) | 19 | (6.8) | |
| **Hypertension** | | | | | 0.198 |
| No | 276 | (98.9) | 230 | (97.0) | |
| Yes | 3 | (1.1) | 7 | (3.0) | |
| **Maternal haemoglobin** | | | | | 0.429 |
| Normal | 249 | (84.7) | 221 | (82.2) | |
| Anaemic | 45 | (15.3) | 48 | (17.8) | |
| **Sex of baby** | | | | | 1.000 |
| Female | 151 | (48.7) | 136 | (48.6) | |
| Male | 159 | (51.3) | 144 | (51.4) | |
| **Received curative treatment for malaria infection** | | | | | 0.810 |
| No | 269 | (86.8) | 239 | (86.0) | |
| Yes | 41 | (13.2) | 39 | (14.0) | |
| **Received curative treatment for any STI/RTI** | | | | | 0.038 |
| Untreated | 291 | (93.9) | 249 | (88.9) | |
| Treated | 19 | (6.1) | 31 | (11.1) | |

* Age is shown as the median value with the interquartile range in parentheses. *P*-values are from Wilcoxon rank sum test (continuous variables) or Fisher's exact test (categorical variables). PCR = polymerase chain reaction

**Table 3.10 Adverse birth outcomes: 0-1 dose vs. 2 doses vs. ≥ 3 doses of IPTp-SP**

| Birth outcome | No. women | Outcomes | Unadjusted OR | 95% CI | Adjusted OR[1] | 95% CI | *P*-value[2] |
|---|---|---|---|---|---|---|---|
| **Any adverse outcome** | | | | | | | |
| 0-1 dose | 126 | 58 | 1.00 | | 1.00 | | **0.002** |
| 2 doses | 310 | 108 | 0.63 | 0.41, 0.96 | 0.55 | **0.36, 0.86** | |
| ≥ 3 doses | 280 | 84 | 0.50 | 0.33, 0.78 | 0.43 | **0.27, 0.68** | |
| **Stillbirth** | | | | | | | |
| 0-1 dose | 126 | 4 | 1.00 | | 1.00 | | 0.143 |
| 2 doses | 310 | 2 | 0.20 | 0.04, 1.10 | 0.21 | 0.04, 1.19 | |
| ≥ 3 doses | 280 | 6 | 0.67 | 0.19, 2.41 | 0.68 | 0.18, 2.57 | |
| **Low birth weight** | | | | | | | |
| 0-1 dose | 126 | 32 | 1.00 | | 1.00 | | 0.261 |
| 2 doses | 310 | 67 | 0.80 | 0.49, 1.30 | 0.71 | 0.42, 1.19 | |
| ≥ 3 doses | 280 | 57 | 0.74 | 0.45, 1.22 | 0.64 | 0.37, 1.09 | |
| **Preterm delivery** | | | | | | | |
| 0-1 dose | 126 | 50 | 1.00 | | 1.00 | | **<0.001** |
| 2 doses | 310 | 71 | 0.45 | 0.29, 0.71 | 0.42 | **0.27, 0.67** | |
| ≥ 3 doses | 280 | 37 | 0.23 | 0.14, 0.38 | 0.21 | **0.13, 0.35** | |
| **Intrauterine growth retardation** | | | | | | | |
| 0-1 dose | 126 | 7 | 1.00 | | 1.00 | | 0.318 |
| 2 doses | 310 | 34 | 1.64 | 0.70, 3.87 | 1.55 | 0.64, 3.77 | |
| ≥ 3 doses | 280 | 43 | 2.12 | 0.91, 4.93 | 1.88 | 0.78, 4.54 | |

IPTp-SP = Intermittent preventive treatment of malaria in pregnancy using sulphadoxine-pyrimethamine; CI = Confidence Interval; OR = Odds Ratio

[1]Adjusted for sexually transmitted and reproductive tract co-infection, gravidae and HIV co-infection.

[2]*P*-value for likelihood ratio test

Note: Confidence intervals that do not overlap the null value of OR = 1 are shown in bold.

Women who had malaria only at enrolment and received ≥ 2 doses of versus 0-1, had 76% lower odds of any adverse birth outcome (OR, 0.24; 95% 0.09, 0.66). Similarly, women who had *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis* at enrolment and received ≥ 2 doses of versus 0-1, had 92% lower odds of any adverse birth outcome (OR, 0.08; 95% CI, 0.01, 0.64). Participants with neither a malaria infection nor STIs/RTIs and who received ≥ 2 doses, rather than 0-1 dose, had 73% fewer adverse birth outcomes (OR 0.27; 95% CI 0.11, 0.68). Similarly, women with no malaria infection nor STIs/RTIs and who received ≥ 2 doses had the odds of LBW reduced by 76% (OR 0.24; 95% CI 0.08, 0.68).

Women who had malaria only at enrolment and received ≥ 2 doses of versus 0-1, had 81% lower odds of preterm birth (OR,0.19; 95% CI, 0.07, 0.53); women who had malaria plus *T. vaginalis* and/or bacterial vaginosis at enrolment and received ≥ 2 doses of versus 0-1, had 55% lower odds of preterm birth (OR, 0.45; 95% CI, 0.21, 0.97); women who had *N. gonorrhoeae* or *C. trachomatis* at enrolment and received ≥ 2 doses of versus 0-1, had 93% lower odds of preterm birth (OR, 0.07; 95% CI, 0.01, 0.73); finally, women who had neither malaria nor any curable STI/RTI at enrolment and received ≥ 2 doses of versus 0-1, had 80% lower odds of preterm birth (OR, 0.20; 95% CI, 0.07, 0.54).

Although less dramatic, analyses of ≥ 3 doses versus 2 doses suggest that this dose-response effect continued as shown in Table 3.11. There appeared to be a trend of protection in 17 of 31 infection categories conferred by ≥ 3 doses verse 2 doses. This protection, however, was only significant in two infection categories in Table 3.12. Women who had malaria plus *T. vaginalis* and/or bacterial vaginosis at enrolment and

received ≥3 doses vs 2 doses had 67% lower odds of preterm birth (OR, 0.33; 95% CI, 0.15, 0.73); women who had *T. vaginalis* and/or bacterial vaginosis at enrolment and received ≥3 doses vs 2 doses had 66% lower odds of preterm birth (OR, 0.34; 95% CI, 0.13, 0.94).

**Table 3.11 Infections and adverse birth outcomes: 0-1 doses vs. ≥ 2 doses IPTp-SP**

| Adverse birth outcomes<br>*Categories of maternal infection* | 0-1 dose IPTp-SP | | ≥ 2 doses IPTp-SP | | Crude OR | 95% CI | Adjusted OR[1] | 95% CI |
|---|---|---|---|---|---|---|---|---|
| | No. women | No. outcomes | No. women | No. outcomes | | | | |
| **Any adverse outcome** | | | | | | | | |
| *Malaria only* | 20 | 13 | 129 | 41 | 0.25 | 0.09, 0.88 | **0.24** | **0.09, 0.66** |
| *Malaria and NG and/or CT* | 3 | 1 | 27 | 11 | 1.38 | 0.11, 17.09 | 1.17 | 0.09, 15.89 |
| *Malaria and TV and/or BV* | 38 | 15 | 182 | 67 | 0.89 | 0.44, 1.83 | 0.96 | 0.45, 2.02 |
| *Syphilis and any other infection(s)* | 1 | 1 | 17 | 7 | 0.80 | 0.00, 31.20 | 0.80 | 0.00, 31.20 |
| *NG and/or CT only* | 6 | 4 | 14 | 2 | 0.08 | 0.01, 0.80 | **0.08** | **0.01, 0.64** |
| *TV and/or BV only* | 32 | 12 | 124 | 42 | 0.85 | 0.38, 1.91 | 0.72 | 0.32, 1.65 |
| *No identified infection* | 26 | 12 | 97 | 22 | 0.34 | 0.14, 0.85 | **0.27** | **0.11, 0.68** |
| **Stillbirth** | | | | | | | | |
| *Malaria only* | 20 | 1 | 129 | 1 | 0.15 | 0.01, 2.54 | 0.15 | 0.01, 2.54 |
| *Malaria and NG and/or CT* | 3 | 0 | 27 | 0 | NA | NA | NA | NA |
| *Malaria and TV and/or BV* | 38 | 0 | 182 | 3 | 0.81 | 0.09, Inf | 0.81 | 0.09, Inf |
| *Syphilis and any other infection(s)* | 1 | 1 | 17 | 0 | 0.06 | 0.00, 2.29 | 0.06 | 0.00, 2.29 |
| *NG and/or CT only* | 6 | 0 | 14 | 0 | NA | NA | NA | NA |
| *TV and/or BV only* | 32 | 1 | 124 | 3 | 0.76 | 0.07, 7.86 | 0.76 | 0.07, 7.86 |
| *No identified infection* | 26 | 1 | 97 | 1 | 0.26 | 0.02, 4.31 | 0.22 | 0.01, 3.79 |
| **Low birth weight** | | | | | | | | |
| *Malaria only* | 20 | 6 | 129 | 25 | 0.56 | 0.20, 1.60 | 0.59 | 0.19, 1.82 |
| *Malaria and NG and/or CT* | 3 | 1 | 27 | 7 | 0.7 | 0.05, 8.97 | 0.49 | 0.03, 7.35 |
| *Malaria and TV and/or BV* | 38 | 10 | 182 | 46 | 0.95 | 0.43, 2.10 | 1.08 | 0.46, 2.54 |
| *Syphilis and any other infection(s)* | 1 | 0 | 17 | 5 | NA | NA | NA | NA |
| *NG and/or CT only* | 6 | 2 | 14 | 1 | 0.15 | 0.01, 2.18 | 0.12 | 0.01, 1.90 |
| *TV and/or BV only* | 32 | 5 | 124 | 27 | 1.5 | 0.53, 4.27 | 1.22 | 0.41, 3.59 |
| *No identified infection* | 26 | 8 | 97 | 13 | 0.35 | 0.13, 0.96 | **0.24** | **0.08, 0.68** |

**Table 3.11 Infections and adverse birth outcomes: 0-1 doses vs. ≥ 2 doses IPTp-SP (continued)**

| Adverse birth outcomes<br>*Categories of maternal infection* | 0-1 dose IPTp-SP | | ≥ 2 doses IPTp-SP | | Crude OR | 95% CI | Adjusted OR[1] | 95% CI |
|---|---|---|---|---|---|---|---|---|
| | No. outcomes | No. women | No. outcomes | No. outcomes | | | | |
| **Preterm delivery** | | | | | | | | |
| *Malaria only* | 20 | 10 | 129 | 21 | 0.19 | 0.07, 0.53 | **0.19** | **0.07, 0.53** |
| *Malaria and NG and/or CT* | 3 | 0 | 27 | 6 | NA | NA | NA | NA |
| *Malaria and TV and/or BV* | 38 | 14 | 182 | 37 | 0.44 | 0.21, 0.93 | **0.45** | **0.21, 0.97** |
| *Syphilis and any other infection(s)* | 1 | 1 | 17 | 5 | 0.50 | 0.00, 19.50 | 0.50 | 0.00, 19.50 |
| *NG and/or CT only* | 6 | 4 | 14 | 2 | 0.08 | 0.01, 0.80 | **0.07** | **0.01, 0.73** |
| *TV and/or BV only* | 32 | 11 | 124 | 25 | 0.48 | 0.21, 1.13 | 0.43 | 0.18, 1.03 |
| *No identified infection* | 26 | 10 | 97 | 12 | 0.23 | 0.08, 0.61 | **0.20** | **0.07, 0.54** |
| **Intrauterine growth retardation** | | | | | | | | |
| *Malaria only* | 20 | 2 | 129 | 18 | 0.8 | 0.16, 4.08 | 0.75 | 0.14, 4.05 |
| *Malaria and NG and/or CT* | 3 | 1 | 27 | 5 | 0.62 | 0.05, 8.43 | 0.54 | 0.03, 8.43 |
| *Malaria and TV and/or BV* | 38 | 1 | 182 | 27 | 5.35 | 0.69, 41.40 | 6.11 | 0.76, 49.13 |
| *Syphilis and any other infection(s)* | 1 | 0 | 17 | 2 | NA | NA | NA | NA |
| *NG and/or CT only* | 6 | 0 | 14 | 0 | NA | NA | NA | NA |
| *TV and/or BV only* | 32 | 1 | 124 | 16 | 3.81 | 0.48, 30.44 | 3.13 | 0.38, 25.68 |
| *No identified infection* | 26 | 2 | 97 | 9 | 0.83 | 0.16, 4.25 | 0.66 | 0.12, 3.57 |

IPTp-SP = intermittent preventive treatment of malaria in pregnancy using sulphadoxine-pyrimethamine; CI = Confidence Interval; OR = Odds Ratio; NG = *Neisseria gonorrhoeae*; CT = *Chlamydia trachomatis*; TV = *Trichomonas vaginalis*; BV = bacterial vaginosis; N/A = not applicable is used where there are no observations in the reference group (0-1 doses); Inf. = infinity is used where the sub-sample of outcomes observed is too small to produce an outer limit with certainty.

[1]Adjusted for sexually transmitted and reproductive tract co-infection, gravidae and HIV co-infection.

[2]Syphilis and any other infection(s) refers to any other curable sexually transmitted or reproductive tract infection and/or malaria

Notes: (1) Confidence intervals that do not overlap the null value of OR = 1 are shown in bold; (2) Syphilis and co-infection describes pregnant women who tested positive for syphilis using rapid plasma regain assays and were also infected with malaria and/or another curable STI/RTI.

**Table 3.12 Infections and adverse birth outcomes: 2 doses vs. ≥ 3 doses IPTp-SP**

| Adverse birth outcomes<br>*Categories of maternal infection* | 2 doses IPTp-SP | | ≥ 3 doses IPTp-SP | | Crude OR | 95% CI | Adjusted OR[1] | 95% CI |
|---|---|---|---|---|---|---|---|---|
| | No. women | No. outcomes | No. women | No. outcomes | | | | |
| **Any adverse outcome** | | | | | | | | |
| *Malaria only* | 66 | 23 | 63 | 18 | 0.75 | 0.35, 1.58 | 0.72 | 0.33, 1.56 |
| *Malaria and NG and/or CT* | 18 | 7 | 9 | 4 | 1.26 | 0.25, 6.36 | 0.93 | 0.17, 5.16 |
| *Malaria and TV and/or BV* | 86 | 36 | 96 | 31 | 0.66 | 0.36, 1.21 | 0.66 | 0.35, 1.24 |
| *Syphilis and any other infection(s)* | 7 | 3 | 10 | 4 | 0.89 | 0.13, 6.31 | 0.5 | 0.07, 3.72 |
| *NG and/or CT only* | 12 | 1 | 2 | 1 | 11 | 0.35, 345.05 | 16.39 | 0.50, 541.53 |
| *TV and/or BV only* | 71 | 27 | 53 | 15 | 0.64 | 0.30, 1.38 | 0.64 | 0.29, 1.41 |
| *No identified infection* | 50 | 11 | 47 | 11 | 1.08 | 0.42, 2.80 | 1.28 | 0.48, 3.43 |
| **Stillbirth** | | | | | | | | |
| *Malaria only* | 66 | 1 | 63 | 0 | 1.03 | 0.00, 40.32 | 1.03 | 0.00, 40.32 |
| *Malaria and NG and/or CT* | 18 | 0 | 9 | 0 | N/A | - | N/A | - |
| *Malaria and TV and/or BV* | 86 | 1 | 96 | 2 | 1.81 | 0.09, 107.93 | 1.81 | 0.09, 107.93 |
| *Syphilis and any other infection(s)* | 7 | 0 | 10 | 0 | N/A | - | N/A | - |
| *NG and/or CT only* | 12 | 0 | 2 | 0 | N/A | - | N/A | - |
| *TV and/or BV only* | 71 | 0 | 53 | 3 | 5.32 | 0.56, Inf | 5.32 | 0.56, Inf |
| *No identified infection* | 50 | 0 | 47 | 1 | 1.81 | 0.16, 20.30 | 2.18 | 0.19, 25.65 |
| **Low birth weight** | | | | | | | | |
| *Malaria only* | 66 | 14 | 63 | 11 | 0.79 | 0.33, 1.89 | 0.74 | 0.30, 1.86 |
| *Malaria and NG and/or CT* | 18 | 5 | 9 | 2 | 0.74 | 0.11, 4.87 | 0.49 | 0.07, 3.62 |
| *Malaria and TV and/or BV* | 86 | 23 | 96 | 23 | 0.86 | 0.44, 1.68 | 0.91 | 0.45, 1.86 |
| *Syphilis and any other infection(s)* | 7 | 2 | 10 | 3 | 1.07 | 0.13, 8.98 | 0.53 | 0.06, 4.87 |
| *NG and/or CT only* | 12 | 1 | 2 | 0 | 6.00 | 0.00, 234 | 6.00 | 0.00, 234 |
| *TV and/or BV only* | 71 | 16 | 53 | 11 | 0.90 | 0.38, 2.14 | 0.90 | 0.38, 2.14 |
| *No identified infection* | 50 | 6 | 47 | 7 | 1.28 | 0.40, 4.14 | 1.59 | 0.47, 5.40 |

**Table 3.12 Infections and adverse birth outcomes: 2 doses vs. ≥ 3 doses IPTp-SP (continued)**

| Adverse birth outcomes<br>*Categories of maternal infection* | 2 doses IPTp-SP | | ≥ 3 doses IPTp-SP | | Crude OR | 95% CI | Adjusted OR[1] | 95% CI |
|---|---|---|---|---|---|---|---|---|
| | No. women | No. outcomes | No. women | No. outcomes | | | | |
| **Preterm delivery** | | | | | | | | |
| *Malaria only* | 66 | 13 | 63 | 8 | 0.59 | 0.23, 1.55 | 0.59 | 0.22, 1.54 |
| *Malaria and NG and/or CT* | 18 | 4 | 9 | 2 | 1.00 | 0.15, 6.85 | 0.83 | 0.12, 5.82 |
| *Malaria and TV and/or BV* | 86 | 25 | 96 | 12 | 0.34 | 0.16, 0.74 | **0.33** | **0.15, 0.73** |
| *Syphilis and any other infection(s)* | 7 | 3 | 10 | 2 | 0.33 | 0.04, 2.87 | 0.23 | 0.03, 2.06 |
| *NG and/or CT only* | 12 | 1 | 2 | 1 | 11 | 0.35, 345.06 | 14.4 | 0.45, 463.93 |
| *TV and/or BV only* | 71 | 19 | 53 | 6 | 0.35 | 0.13, 0.95 | **0.34** | **0.13, 0.94** |
| *No identified infection* | 50 | 6 | 47 | 6 | 1.07 | 0.32, 3.59 | 1.19 | 0.35, 4.03 |
| **Intrauterine growth retardation** | | | | | | | | |
| *Malaria only* | 66 | 9 | 63 | 9 | 0.96 | 0.35, 2.63 | 1.01 | 0.35, 2.89 |
| *Malaria and NG and/or CT* | 18 | 3 | 9 | 2 | 1.47 | 0.18, 11.72 | 0.64 | 0.07, 5.74 |
| *Malaria and TV and/or BV* | 86 | 9 | 96 | 18 | 1.52 | 0.63, 3.65 | 1.54 | 0.61, 3.88 |
| *Syphilis and any other infection(s)* | 7 | 0 | 10 | 2 | 1.31 | 0.09, Inf | 1.31 | 0.09, Inf |
| *NG and/or CT only* | 12 | 0 | 2 | 0 | N/A | - | N/A | - |
| *TV and/or BV only* | 71 | 8 | 53 | 8 | 1.15 | 0.40, 3.36 | 1.28 | 0.42, 3.89 |
| *No identified infection* | 50 | 5 | 47 | 4 | 0.84 | 0.21, 3.38 | 0.88 | 0.21, 3.70 |

IPTp-SP = intermittent preventive treatment of malaria in pregnancy using sulphadoxine-pyrimethamine; CI = Confidence Interval; OR = Odds Ratio; NG = *Neisseria gonorrhoeae*; CT = *Chlamydia trachomatis*; TV = *Trichomonas vaginalis*; BV = bacterial vaginosis; N/A = not applicable is used where there are no observations in the reference group (0-1 doses); Inf. = infinity is used where the sub-sample of outcomes observed is too small to produce an outer limit with certainty.

[1]Adjusted for sexually transmitted and reproductive tract co-infection, gravidae and HIV co-infection.

[2]Syphilis and any other infection(s) refers to any other curable sexually transmitted or reproductive tract infection and/or malaria

Notes: (1) Confidence intervals that do not overlap the null value of OR = 1 are shown in bold; (2) Syphilis and co-infection describes pregnant women who tested positive for syphilis using rapid plasma regain assays and were also infected with malaria and/or another curable STI/RTI.

## Discussion

For more than a decade, members of the malaria in pregnancy research community have attempted to identify safe alternatives to SP for use in IPTp because of known parasite resistance to the therapy. Several factors have complicated this task including the provision of ITNs to pregnant women as never before that have altered transmission dynamics. Evidence from Zambia – suggesting that IPTp-SP protects against adverse birth outcomes among women with malaria as well as curable STIs/RTIs – may help to explain why clinical trials to date have not yielded a clear superior alternative to SP.

One of the most interesting findings in this analysis was that women who received $\geq$ 2 doses and had neither malaria nor curable STIs/RTIs were more protected against any adverse birth outcome, LBW, and preterm delivery, compared to recipients of 0-1 dose. No additional benefit was conferred against non-malaria and non-STI/RTI causes of adverse birth outcomes when $\geq$ 3 doses were administered in this setting.

Although there were no statistically significant differences across IPTp-SP exposure groups in the baseline characteristics specific to malaria infection and curable STIs/RTIs, there were a few notable imbalances in sub-groups. Confounding, however, is not inherently produced by imbalances in size of groups. Rather, confounding is determined by comparing the difference between crude odds ratios and adjusted odds ratios specific to a variable of interest.[206] In this way, three confounders were identified in three analyses, one in each:

(i) Hypertension at enrolment or delivery (change in crude OR = 13.39%) in the stillbirth analysis of Table 3.2 (pages 88-89);

(ii) Prior preterm birth (change in crude OR = 14.04%) in the preterm delivery analysis of Table 3.4 (pages 92-93); and

(iii) Prior miscarriage (change in crude OR = 25.73%) in the intrauterine growth retardation analysis of Table 3.5 (pages 94-95).

Amongst women given 0-1 dose versus $\geq$ 2 dose in Table 3.6, there were three statistically significant differences in group size: the wealth quintiles ($P$ = 0.048), (ii) sex of the baby ($P$ = 0.008), and (iii) malaria infection ($P$ = 0.047). The differences in wealth quintiles may not have had a consequential effect on outcomes because, upon closer review of the individual quintiles, the imbalance appears to have straddled the two exposure groups. The lowest (poorest) quintile had proportionately more women in the $\geq$ 2 dose-group; the second and third wealth quintiles had more women from the 0-1 dose-group; the fourth wealth quintile had more women from the $\geq$ 2 dose-group; and the highest (wealthiest) quintile has near equivalent representation from the two dose-groups. Another difference was that fewer males, compared to females, were in the 0-1 dose exposure group, 38.1% (n = 48) to 61.9% (n = 78), respectively, relative to 51.4% (n = 303) males and 48.6% (n = 287) females born to mothers who received $\geq$ 2 doses. However, this difference in the 0-1 dose-group did not influence the overall protective effect of IPTp-SP.

As for malaria infection at enrolment, 10% more women were parasitaemic who went on to receive $\geq$ 2 doses of IPTp-SP, compared to recipients of 0-1 dose (59.3% [n = 346] versus 49.2% [n = 62]; ($P$ = 0.047). This finding, interestingly, reflects the epidemiology

of malaria during pregnancy. Amongst women enrolled between 8 and 13 gestational weeks, 36.1% (95% CI: 22.5, 52.5) had peripheral parasitaemia. These same women went onto receive a mean of 2.6 doses of IPTp-SP over the course of their pregnancies. However, peripheral malaria was lower, 33.5% (95% CI: 29.1, 38.3), amongst participants who were enrolled between 14 and 20 gestational weeks and subsequently received a mean of 2.4 doses of IPTp-SP during their pregnancies. These two trends of parasitaemia and the number of IPTp-SP doses continued downward amongst women who were between 21 and 26 gestational weeks, and 27 to 31 gestational weeks, at their time of enrolment. Peripheral parasitaemia was 31.7% (95% CI: 27.5, 36.2) and 27.6% (95% CI: 21.9, 34.2), and the mean number of IPTp-SP doses administered until delivery was 2.2 and 1.8, respectively. In this analysis, parasite load was highest amongst women enrolled in gestational week 15 with a mean density of 4,752 parasites per microliter of blood. The peak parasite density observed was consistent with the known epidemiology of malaria infection during pregnancy. Prior reports suggest that peripheral parasitaemia peaks between weeks nine and 16, and tapers to term.[160,169] Thus, pregnant women who sought ANC services earlier in pregnancy were more likely to have a malaria infection and they were also more likely to receive more doses of IPTp-SP during pregnancy for having engaged the health system earlier in pregnancy.

The last notable imbalance was in the proportion of pregnant women from households where indoor residual spraying had been applied in the previous 12 months (*P*-value = 0.002); 17.1% (n = 51) of women in the 2-dose group compared to 28.4% (n = 76) in

the ≥ 3-dose group. Indoor residual spraying has reduced the incidence of infections by 54% over an 11–month period amongst children under the age of five in an area of stable transmission.[207] Despite evidence of protection conferred by household spraying, the pregnant women resident in homes that had been sprayed and who went on to receive ≥ 3 doses of IPTp-SP had higher levels of parasitaemia at booking, 62.9% (n = 175), compared to women in the 2-dose group, 56.1% (n = 171), as measured by polymerase chain reaction.

Observations from this analysis are biologically plausible and compelling. Arguably, because only six women of the 126 in the 0-1 dose-group received no doses in study, the actual protective effect of IPTp-SP against malaria and curable STIs/RTIs is likely higher than observed levels. It is worth noting that the number of ANC visits were not included in the survey and, therefore, could not be controlled for in this analysis. Being the case, it is difficult to draw inferences related to a general ANC visit effect. With that said, there was no apparent confounding across treatment groups in three key variables that reflect use of ANC services: (1) STI/RTI treatment during pregnancy; (2) antibiotic treatment during the pregnancy; and (3) malaria treatment during the pregnancy.

Results from Zambia are insufficient to make broader policy or programmatic decisions. Curable STIs/RTIs were just measured once at booking; there was no test of cure. Some curable STIs/RTIs can be self-limiting which could, in part, explain observations. Thus, until a candidate replacement for SP has been able to demonstrate superior protection against malaria infection and curable STIs/RTIs, and attributable adverse birth outcomes, policymakers and programme managers should

continue to champion the scale up IPTp-SP as part of the ANC package. Moreover, it is difficult to separate the protective effect of IPTp-SP attributable to malaria versus curable STIs/RTIs in the absence of continuous parasite sampling throughout pregnancy. It is possible that many women were exposed to malaria infection repeatedly during pregnancy, irrespective of their STI/RTI status, and that the benefits of receiving more doses of IPTp-SP translated into having greater protection against the effects of malaria in pregnancy than curable STIs/RTIs. Repeated malaria infection may also help to explain the dose-response protective effect of IPTp-SP against adverse birth outcomes observed amongst pregnant women who had neither malaria infection nor STIs/RTIs at enrolment. These women could have been infected by malaria parasites later in pregnancy, but were protected against adverse birth outcomes attributable to malaria if they received more doses of IPTp-SP compared to fewer doses.

## Q 4. Prevalence of malaria and curable STIs/RTIs in pregnancy

### Methods

Surprisingly, the prevalence of malaria infection amongst pregnant women in sub-Saharan Africa has not been estimated recently. Brabin *et al.* summarised malaria infection data prior to 1980[160] and Steketee *et al.* published a review of data from 20 studies across eight countries conducted between 1985 and 2000.[18] Neither review contains data from 2000 onward, nor applied the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) that were published in 2009 to create standard methods for systematic reviews.[167] There does not appear to have been any prior attempt to calculate pooled prevalence estimates for curable

STIs/RTIs amongst pregnant women in sub-Saharan Africa. Thus, a systematic review was conducted of malaria infection and curable STIs/RTIs amongst pregnant women attending ANC facilities from 1990 to 2011 in sub-Saharan Africa using medical subject headings and free text terms shown in Table 4.a. Databases included PubMed, Medline, and EMBASE, as well as the WHO International Clinical Trials Registry and reference lists from the identified publications.

**Table 4.a Medical subject headings and free text terms**

| Infection | Medical search headings | | Free text terms |
|---|---|---|---|
| **Syphilis** | *Treponema pallidum* OR Syphilis AND "Pregnant Women" AND "Africa South of the Sahara" | Topic=("Syphilis" OR "*Treponema pallidum*") AND Topic=(Prenatal OR Antenatal OR Pregnant or Pregnancy) OR Topic=(women) AND Topic=(Africa) | *Treponema pallidum* AND Pregnant AND Africa<br>*Treponema pallidum* AND Pregnancy AND Africa<br>Syphilis AND Pregnant AND Africa<br>Syphilis AND Pregnancy AND Africa<br>Screen AND Syphilis AND Pregnant AND Africa<br>Screen AND Syphilis AND Pregnancy AND Africa<br>Test AND Syphilis AND Pregnant AND Africa<br>Test AND Syphilis AND Pregnancy AND Africa |
| ***Neisseria gonorrhoeae*** | *Neisseria gonorrhoeae* OR Gonorrhea OR Gonorrhoea AND Pregnant Women AND "Africa South of the Sahara" | Topic=("Neisseria gonorrhoeae") OR Topic=(Gonorrhea) OR Topic=(Gonorrhoea) AND Topic= (Prenatal OR Antenatal OR Pregnant OR Pregnancy) OR Topic=(women) AND Topic=(Africa) | *Neisseria gonorrhoeae* AND Pregnant AND Africa<br>*Neisseria gonorrhoeae* AND Pregnancy AND Africa<br>Gonorrhoea AND Pregnant AND Africa<br>Gonorrhoea AND Pregnancy AND Africa<br>Gonorrhea AND Pregnant AND Africa<br>Gonorrhea AND Pregnancy AND Africa<br>Screen AND Gonorrhea AND Pregnant AND Africa<br>Screen AND Gonorrhoea AND Pregnant AND Africa<br>Test AND Gonorrhea AND Pregnant AND Africa<br>Test AND Gonorrhoea AND Pregnant AND Africa |
| ***Chlamydia trachomatis*** | *Chlamydia trachomatis* AND Pregnant Women AND "Africa South of the Sahara" | Topic=("Chlamydia") AND Topic= (trachomatis) AND Topic=(Prenatal OR Antenatal OR Pregnant OR Pregnancy) OR Topic=(women) AND Topic=(Africa) | *Chlamydia trachomatis* AND Pregnant AND Africa<br>*Chlamydia trachomatis* AND Pregnancy AND Africa<br>*Chlamydia* AND Pregnant AND Africa<br>*Chlamydia* AND Pregnancy AND Africa<br>Screen AND Chlamydia AND Pregnant AND Africa<br>Screen AND Chlamydia AND Pregnancy AND Africa<br>Test AND Chlamydia AND Pregnant AND Africa<br>Test AND Chlamydia AND Pregnancy AND Africa |

**Table 4. Medical subject headings and free text terms (continued)**

| Infection | Medical search headings | Free text terms | Infection |
|---|---|---|---|
| ***Trichomonas vaginalis*** | *Trichomonas vaginalis* AND Pregnant Women AND "Africa South of the Sahara" | Topic=("*Trichomonas vaginalis* OR Trichomoniasis") AND Topic=(Prenatal OR Antenatal OR Pregnant OR Pregnancy) OR Topic=(women) AND Topic=(Africa) | *Trichomonas vaginalis* AND Pregnant AND Africa<br>*Trichomonas vaginalis* AND Pregnancy AND Africa<br>Trichomoniasis AND Pregnant AND Africa<br>Trichomoniasis AND Pregnancy AND Africa<br>Screen AND Trichomoniasis AND Pregnant AND Africa<br>Screen AND Trichomoniasis AND Pregnancy AND Africa<br>Test AND Trichomoniasis AND Pregnant AND Africa<br>Test AND Trichomoniasis AND Pregnancy AND Africa |
| **Bacterial vaginosis** | Bacterial vaginosis AND Pregnant Women AND "Africa South of the Sahara" | Topic=("Bacterial vaginosis") AND Topic=(Prenatal OR Antenatal OR Pregnant OR Pregnancy OR Pregnant) OR Topic=(women) AND Topic=(Africa) | Bacterial vaginosis AND Pregnant AND Africa<br>Bacterial vaginosis AND Pregnancy AND Africa<br>Screen AND Bacterial vaginosis AND Pregnant AND Africa<br>Screen AND Bacterial vaginosis AND Pregnancy AND Africa<br>Test AND Bacterial vaginosis AND Pregnant AND Africa<br>Test AND Bacterial vaginosis AND Pregnancy AND Africa |
| **Malaria** | Malaria AND Pregnant Women AND "Africa South of the Sahara" | Topic=("Malaria") AND Topic=(Prenatal OR Antenatal OR Pregnancy OR Pregnant) OR Topic=(women) AND Topic=(Africa) | Malaria AND Pregnant AND Africa<br>Malaria AND Pregnancy AND Africa<br>Screen AND Malaria AND Pregnant AND Africa<br>Screen AND Malaria AND Pregnancy AND Africa<br>Test AND Malaria AND Pregnant AND Africa<br>Test AND Malaria AND Pregnancy AND Africa |

If two or more prevalence estimates had been reported – whether a study of comparative diagnostics, or use of the same diagnostic method on multiple occasions, or the subsequent use of a more sensitive assay – the higher/highest measurement was extracted. Studies that selectively enrolled women were excluded, e.g. only pregnant women with HIV infection, studies of commercial sex workers, studies of women seeking treatment at STI and/or family planning facilities, or studies of non-pregnant women tested in community-based surveys. Prevalence estimates from these populations may overstate the burden of disease amongst pregnant women at ANC facilities. Studies from South Africa were also excluded where malaria is no longer endemic, as well as multi-year studies that included pre-1990 data. Records retrieved were all in English, although several abstracts had been translated from Chinese, Danish, Dutch, French, German, Italian, Portuguese and Swedish languages. Data were abstracted without blinding author names or publication titles. Point prevalence estimates and 95% CIs were calculated for each study using the data that had been published. Then a standard method for correcting errors of magnitude with the known sensitivity and specificity of individual assays was applied to increase the precision of each point prevalence estimate.[208] This is done using the formula below:

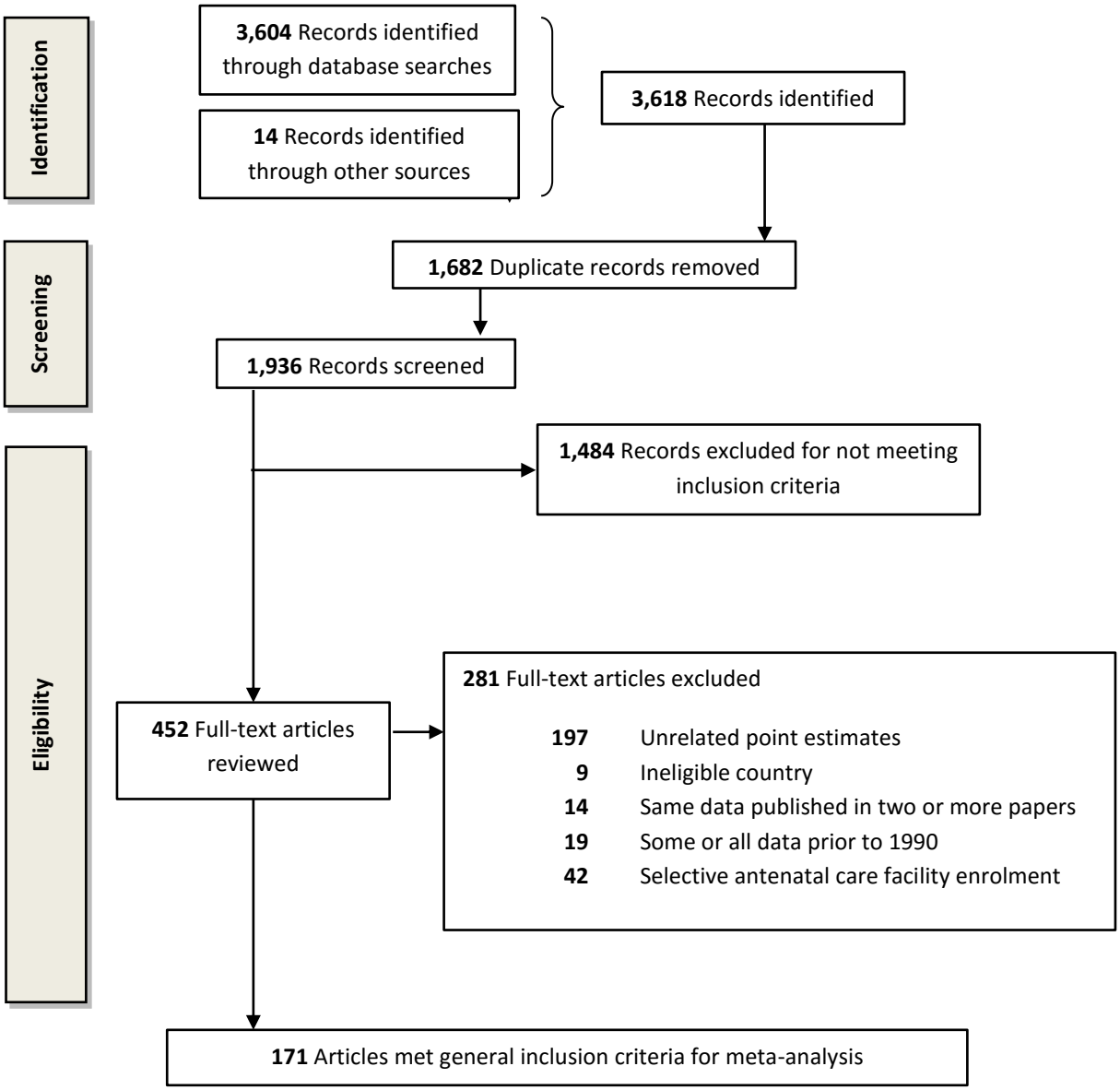
$$Corrected\ Prevalence = \frac{(Observed\ Prevalence + Specificity - 1)}{(Sensitivity + Specificity - 1)}$$

The corrected point prevalence data were then used to generate pooled prevalence estimates and corresponding 95% CIs. Results were stratified by sub-region, East and Southern Africa versus West and Central Africa. Sensitivity analyses were performed by dividing studies into two groups, those conducted from 1990 to 1999, and others from 2000 to 2011, to examine the potential for temporal changes in prevalence.

## Results

In total 171 studies met inclusion criteria, containing a total of 307 individual point prevalence estimates for analysis. Search results are summarised in the PRISMA flowchart of Figure 4.1. Funnel plot analysis suggested there was no evidence of publication bias amongst results for malaria or any of the curable STIs/RTIs. Table 4.1 summarises the pooled prevalence estimates of 1990-2011 and sub-analysis of data from 1990-1999 and 2000-2011. Results that span the period 1990-2011 are presented in Figure 4.1a. and Figure 4.1b. plotted by sub-region along with the lowest and highest point estimates that were reported in the literature. Sub-analysis by infection and sub-region are presented in Figures 4.2 to 4.8 with 'a' corresponding to data from East and Southern Africa and 'b' reflecting studies conducted in West and Central Africa. Details of each study are presented in Tables 4.2 to 4.8 by infection. In all instances, point prevalence estimates have been corrected to account for the variability of precision associated with each diagnostic method used. The published sensitivity and specificity of these assays are summarised in Table 4.9.

**Figure 4.1 PRISMA flowchart for systematic review**



## Table 4.1 Pooled curable STIs/RTIs and prevalence: sub-Saharan Africa

| Sub-region<br>Infection | 1990-2011 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pooled mean prevalence [1] | 95% CI | No. of women positive | No. of women tested | Median positives [2] | Range of study sizes | No. of countries | No. of studies | Range of ANC facilities (per study ) | Heterogeneity |
| **East and Southern Africa** | | | | | | | | | | |
| Syphilis | 4.5 | 3.9, 5.1 | 8,346 | 136,686 | 54 | 85 – 52,405 | 11 | 41 | 1 – 57 | * |
| *Neisseria gonorrhoeae* | 3.7 | 2.8, 4.6 | 626 | 17,220 | 20 | 145 – 9,104 | 7 | 14 | 1 – 13 | 87.5% |
| *Chlamydia trachomatis* | 6.9 | 5.1, 8.6 | 350 | 5,159 | 34 | 151 – 964 | 8 | 10 | 1 – 13 | 85.9% |
| *Trichomonas vaginalis* | 29.1 | 20.9, 37.2 | 5,502 | 28,189 | 86 | 100 – 9,137 | 9 | 18 | 1 – 13 | * |
| Bacterial vaginosis | 50.8 | 43.3, 58.4 | 4,280 | 14,112 | 245 | 100 – 3,046 | 8 | 11 | 1 – 13 | * |
| Peripheral parasitaemia | 32.0 | 25.9, 38.0 | 11,688 | 47,443 | 195 | 86 – 5,093 | 8 | 35 | 1 – 10 | * |
| Placental parasitaemia | 25.8 | 19.7, 31.9 | 1,388 | 6,649 | 86 | 85 – 2,502 | 7 | 12 | 1 – 9 | * |
| **West and Central Africa** | | | | | | | | | | |
| Syphilis | 3.5 | 1.8, 5.2 | 851 | 10,797 | 18 | 205 – 4,100 | 9 | 11 | 1 – 98 | * |
| *Neisseria gonorrhoeae* | 2.7 | 1.7, 3.7 | 73 | 2,737 | 17 | 230 – 1,160 | 8 | 5 | 1 – 4 | 67.2% |
| *Chlamydia trachomatis* | 6.1 | 4.0, 8.3 | 357 | 5,414 | 35 | 261 – 1,160 | 8 | 10 | 1 – 4 | 94.7% |
| *Trichomonas vaginalis* | 17.8 | 12.4, 23.1 | 822 | 9,806 | 57 | 86 – 2,657 | 7 | 12 | 1 – 4 | * |
| Bacterial vaginosis | 37.6 | 18.0, 57.2 | 1,208 | 7,435 | 138 | 350 – 2,657 | 6 | 7 | 1 – 3 | * |
| Peripheral parasitaemia | 38.2 | 32.3, 44.1 | 12,242 | 43,312 | 127 | 26 – 6,370 | 10 | 56 | 1 – 55 | * |
| Placental parasitaemia | 39.9 | 34.2, 45.7 | 4,658 | 27,535 | 168 | 36 – 8,310 | 8 | 23 | 1 – 6 | * |

[1]Note that dividing the total positive diagnoses by the total tested women does not account for diagnostic errors. A standard method of correcting errors of known magnitude has been applied to these data based on the sensitivity and specificity of individual diagnostic tests.
[2]The median number of women with a positive diagnosis.
*Heterogeneity measure >97.0%

## Table 4.1 Pooled curable STIs/RTIs and prevalence: sub-Saharan Africa (continued)

| Sub-region<br>Infection | 1990-1999 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pooled mean prevalence [1] | 95% CI | No. of women positive | No. of women tested | Median positives [2] | Range at study sizes | No. of countries | No. of studies | Range of facilities (per study ) | Heterogeneity |
| **East and Southern Africa** | | | | | | | | | | |
| Syphilis | 6.1 | 5.0, 7.2 | 6,168 | 97,055 | 79 | 85 – 52,405 | 8 | 21 | 1 – 23 | * |
| *Neisseria gonorrhoeae* | 3.7 | 2.7, 4.6 | 547 | 15,446 | 18 | 151 – 9,104 | 6 | 9 | 1 – 12 | 82.5% |
| *Chlamydia trachomatis* | 9.4 | 6.0, 12.7 | 194 | 2,395 | 36 | 151 – 964 | 4 | 5 | 1 – 12 | 88.2% |
| *Trichomonas vaginalis* | 32.8 | 18.6, 47.1 | 4,314 | 17,710 | 74 | 100 – 9,137 | 5 | 10 | 1 – 12 | * |
| Bacterial vaginosis | 51.5 | 35.5, 67.5 | 1,876 | 7,039 | 109 | 100 – 3,046 | 5 | 6 | 1 – 1 | * |
| Peripheral parasitaemia | 34.9 | 25.1, 44.7 | 7,563 | 29,068 | 327 | 102 – 5,093 | 7 | 16 | 1 – 10 | * |
| Placental parasitaemia | 25.2 | 16.0, 34.3 | 588 | 3,271 | 124 | 232 – 2,502 | 3 | 3 | 1 | * |
| **West and Central Africa** | | | | | | | | | | |
| Syphilis | 3.8 | 1.5, 6.2 | 773 | 7,106 | 13 | 205 – 4,100 | 6 | 17 | 1 – 4 | * |
| *Neisseria gonorrhoeae* | 3.0 | 1.8, 4.2 | 70 | 2,507 | 18 | 350 – 1,160 | 7 | 4 | 1 – 4 | 67.2% |
| *Chlamydia trachomatis* | 7.5 | 4.6, 10.0 | 339 | 4,632 | 44 | 350 – 1,160 | 7 | 5 | 1 – 4 | 94.7% |
| *Trichomonas vaginalis* | 23.5 | 15.6, 31.5 | 721 | 4,585 | 73 | 86 – 1,160 | 6 | 8 | 1 – 4 | * |
| Bacterial vaginosis | 46.0 | 24.2, 67.8 | 640 | 2,645 | 132 | 350 – 646 | 5 | 5 | 1 – 3 | * |
| Peripheral parasitaemia | 43.4 | 34.1, 52.7 | 5,543 | 16,671 | 178 | 26 – 2,104 | 7 | 19 | 1 – 17 | * |
| Placental parasitaemia | 42.6 | 34.9, 50.3 | 2,445 | 19,582 | 233 | 64 – 8,310 | 6 | 9 | 1 – 2 | * |

[1]Note that dividing the total positive diagnoses by the total tested women does not account for diagnostic errors. A standard method of correcting errors of known magnitude has been applied to these data based on the sensitivity and specificity of individual diagnostic tests.
[2]The median number of women with a positive diagnosis.
*Heterogeneity measure >97.0%

## Table 4.1 Pooled curable STIs/RTIs and prevalence: sub-Saharan Africa (continued)

| Sub-region<br>Infection | 2000-2011 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pooled mean prevalence [1] | 95% CI | No. of women positive | No. of women tested | Median positives[2] | Range at study sizes | No. of countries | No. of studies | Range of facilities (per study ) | Heterogeneity |
| **East and Southern Africa** | | | | | | | | | | |
| Syphilis | 2.9 | 2.1, 3.6 | 2,178 | 39,631 | 32 | 245 – 17,277 | 8 | 17 | 1 – 57 | * |
| *Neisseria gonorrhoeae* | 4.9 | 1.8, 7.9 | 79 | 1,774 | 21 | 145 – 835 | 6 | 4 | 1 – 13 | 91.9% |
| *Chlamydia trachomatis* | 5.2 | 3.4, 7.1 | 156 | 2,764 | 25 | 151 – 835 | 6 | 5 | 1 – 13 | 79.6% |
| *Trichomonas vaginalis* | 24.9 | 18.3, 31.5 | 1,188 | 10,479 | 98 | 151 – 2,917 | 8 | 8 | 1 – 13 | * |
| Bacterial vaginosis | 50.3 | 43.9, 56.7 | 2,404 | 7,073 | 253 | 247 – 2,555 | 6 | 5 | 1 – 13 | * |
| Peripheral parasitaemia | 29.5 | 22.4, 36.5 | 4,125 | 18,375 | 157 | 86 – 2,459 | 8 | 19 | 1 – 9 | * |
| Placental parasitaemia | 26.5 | 16.7, 36.4 | 800 | 3,378 | 58 | 85 – 726 | 3 | 9 | 1 – 9 | * |
| **West and Central Africa** | | | | | | | | | | |
| Syphilis | 2.5 | 0.4, 4.6 | 78 | 3,691 | 21 | 230 – 2,133 | 4 | 5 | 1 – 98 | 88.6% |
| *Neisseria gonorrhoeae* | 1.6 | 0.0, 3.3 | 3 | 230 | 3 | 230 | 3 | 1 | 1 – 1 | NA[3] |
| *Chlamydia trachomatis* | 1.9 | 0.2, 3.5 | 18 | 782 | 9 | 261 – 521 | 2 | 2 | 1 – 2 | 55.8% |
| *Trichomonas vaginalis* | 4.5 | 2.5, 6.6 | 101 | 5,221 | 25 | 201 – 2,657 | 2 | 4 | 1 – 4 | 92.7% |
| Bacterial vaginosis | 16.7 | -12.6, 46.1 | 604 | 4,790 | 302 | 2,133 – 2,657 | 2 | 2 | 1 – 2 | * |
| Peripheral parasitaemia | 35.1 | 28.2, 41.9 | 6,699 | 26,641 | 97 | 38 – 6,370 | 8 | 36 | 1 – 55 | * |
| Placental parasitaemia | 38.0 | 28.4, 47.6 | 2,213 | 7,953 | 84 | 36 – 1,875 | 6 | 15 | 1 – 6 | * |

[1]Note that dividing the total positive diagnoses by the total tested women does not account for diagnostic errors. A standard method of correcting errors of known magnitude has been applied to these data based on the sensitivity and specificity of individual diagnostic tests.
[2]The median number of women with a positive diagnosis.
[3]Not applicable as there is just one study
*Heterogeneity measure >97.0%

**Figure 4.1a Curable STIs/RTIs and malaria infection: East and Southern Africa**

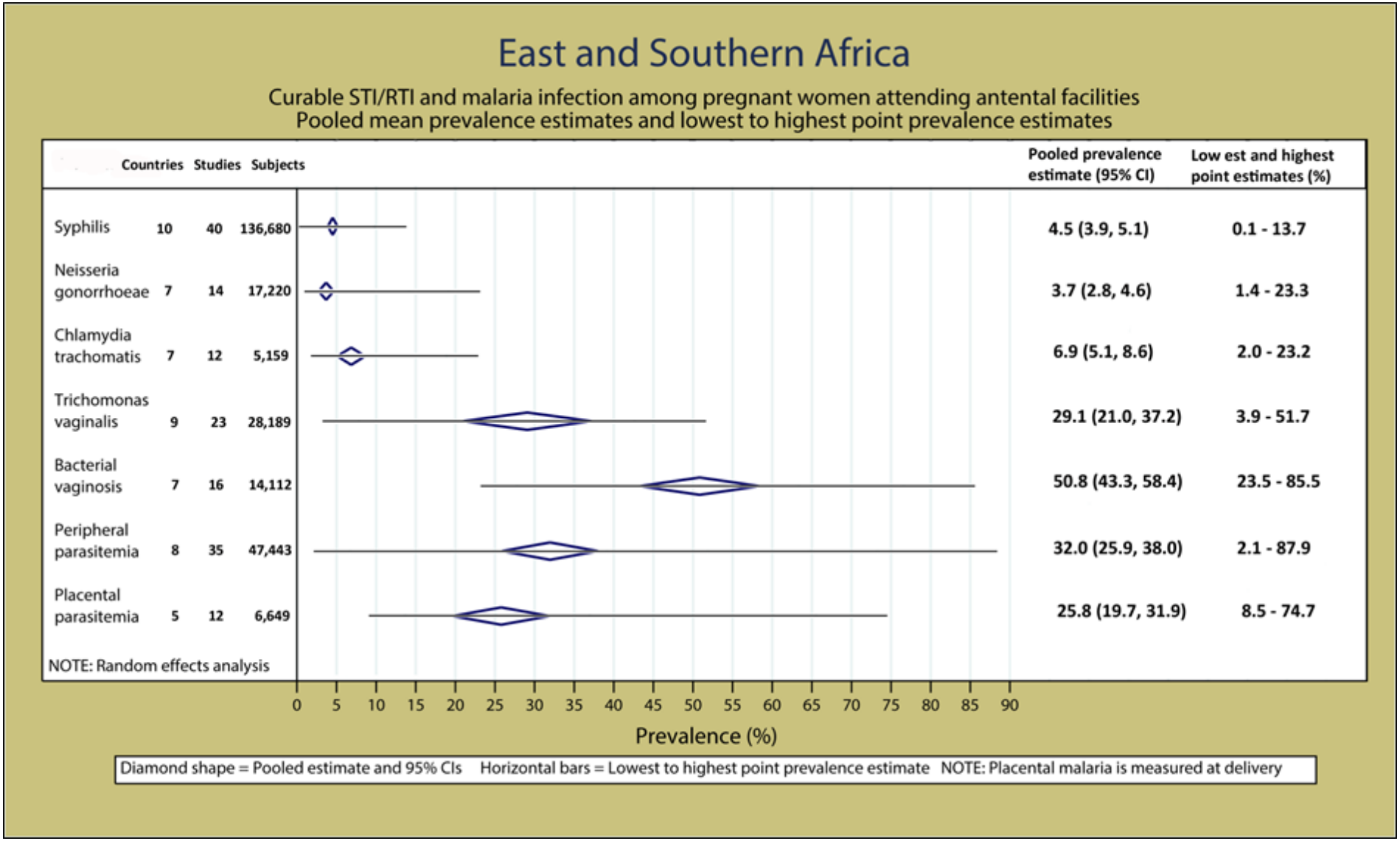
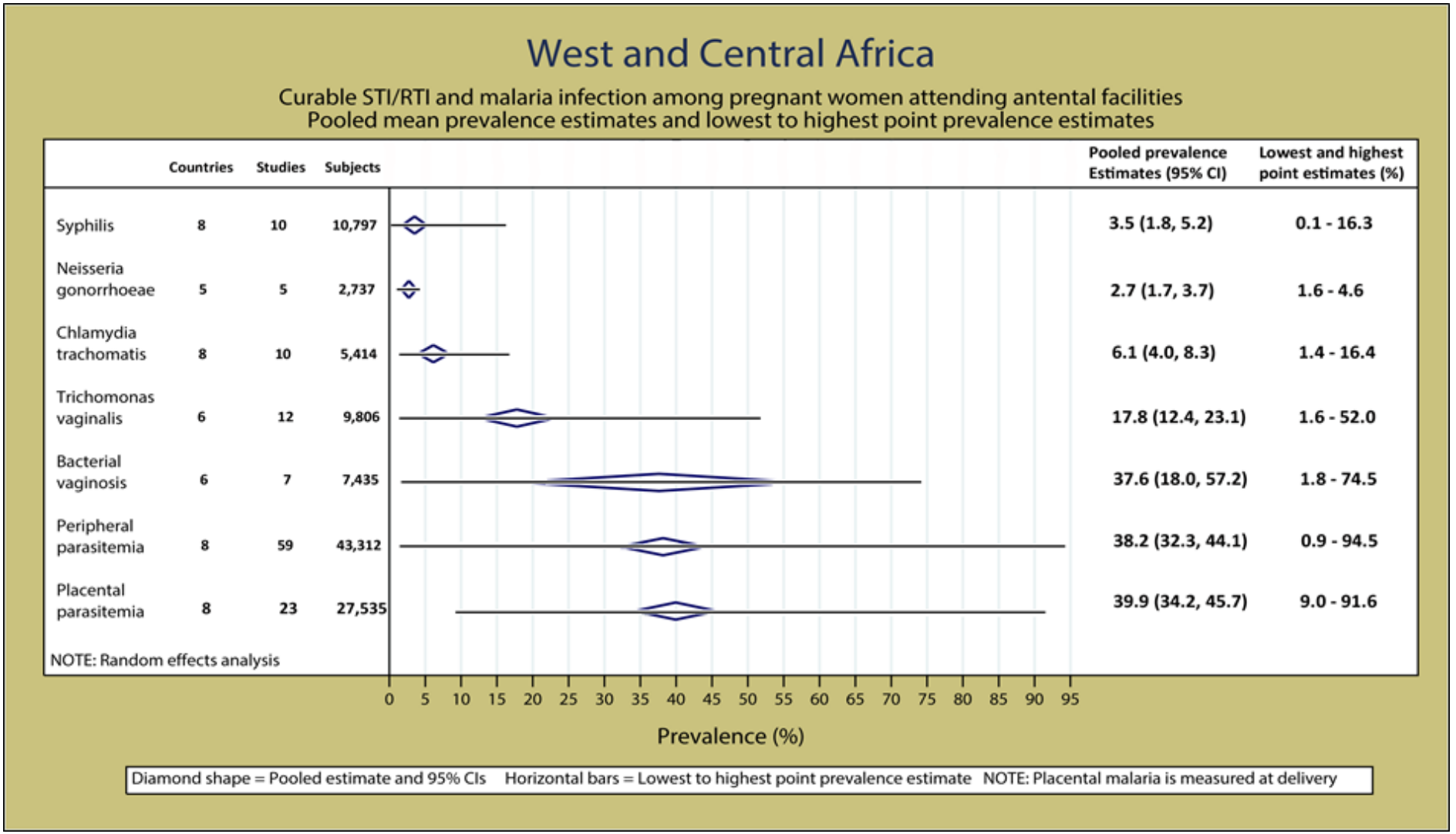


**Figure 4.1b Curable STIs/RTIs and malaria infection: West and Central Africa**



**Syphilis prevalence**

The pooled mean prevalence of syphilis in East and Southern Africa was 4.5% (95% CI: 3.9, 5.1) as shown in Figure 4.2a with the highest estimate, 13.7% (95% CI: 9.0, 18.5), reported from Vilanculos, Mozambique, and based on RPR testing and confirmatory *Treponema pallidum* haemagglutination (TPHA) assays[209] as presented in Table 4.2. In West and Central Africa, the pooled mean prevalence of serological syphilis was 3.5% (95% CI: 1.8, 5.2) as shown in Figure 4.2b. The highest point estimate of 16.3% (95% CI: 15.2, 17.4) was from Yaoundé, Cameroon where TPHA assays were used[210] as presented in Table 4.2.

## Table 4.2 Syphilis point estimates: sub-Saharan Africa

| Site (year study conducted) | | Reference (year of publication) | No. women positive | No. women tested | Uncorrected prevalence (%) | 95% CI | Diagnostic method | Corrected prevalence (%) | 95% CI | No. ANC facilities |
|---|---|---|---|---|---|---|---|---|---|---|
| **East and Southern Africa** | | | | | | | | | | |
| 1990-1999 | Blantyre, Malawi (1990-93) | Taha (1999) [211] | 1001 | 9309 | 10.8 | 10.2, 11.4 | RPR and TPHA | 10.0 | 9.3, 10.6 | 1 |
| | Umzingwane Zimbabwe (1991) | Rutgers (1993) [212] | 197 | 1,433 | 13.8 | 12.0, 15.5 | RPR only | 10.5 | 8.9, 12.0 | 1 |
| | Vilanculos, Mozambique (1991-92) | Vuylsteke (1993) [209] | 29 | 201 | 14.4 | 9.6, 19.3 | RPR and TPHA | 13.7 | 9.0, 18.5 | 1 |
| | Zambizai, Mozambique (1992-93) | Cossa (1994) [213] | 212 | 1,728 | 12.2 | 10.7, 13.8 | RPR and MHA-TP | 11.5 | 10.0, 13.0 | 14 |
| | Nairobi, Kenya (1992-94) | Jenniskens (1995) [214] | 854 | 13,131 | 6.5 | 6.1, 6.9 | RPR only | 2.3 | 2.0, 2.5 | 9 |
| | Mwanza, Tanzania (1992-93) | Mayaud (1995) [215] | 97 | 964 | 10.1 | 8.2, 12.0 | RPR and TPHA | 9.3 | 7.4, 11.1 | 12 |
| | Nairobi, Kenya (1992-97) | Temmerman (1999) [216] | 2,701 | 52,405 | 5.2 | 5.0, 5.3 | RPR only | 0.7 | 0.7, 0.8 | 10 |
| | Dar es Salaam, Tanzania (1993) | Mwakagile (1996) [217] | 31 | 777 | 4.0 | 2.6, 5.4 | RPR and TPHA | 0.1 | 1.8, 4.3 | 1 |
| | Blantyre, Malawi (1993) | Taha (1998) [218] | 264 | 2,161 | 12.2 | 10.8, 13.6 | RPR and TPHA | 11.5 | 9.4, 17.5 | 1 |
| | Debretabor, Ethiopia (1994) | Azeze (1995) [219] | 37 | 270 | 13.7 | 9.6, 17.8 | VDRL only | 13.5 | 9.4, 17.5 | 1 |
| | Nairobi, Kenya (1994) | Thomas (1996) [220] | 9 | 286 | 3.2 | 1.1, 5.2 | RPR and TPHA | 2.2 | 0.5, 3.9 | 1 |
| | Rwandan camp, Tanzania (1994) | Mayaud (1997) [221] | 2 | 100 | 2.0 | -0.7, 4.7 | RPR and TPHA | 1.0 | -1.0, 3.0 | 1 |
| | Mwanza, Tanzania (not reported) | Mayaud (1997) [222] | 173 | 2,380 | 7.3 | 6.2, 8.3 | RPR and TPHA | 6.4 | 5.4, 7.4 | 12 |
| | Mwanza, Tanzania (1994) | Mayaud (1998) [223] | 55 | 660 | 8.3 | 6.2, 10.4 | RPR and TPHA | 7.5 | 5.5, 9.5 | 1 |
| | Blantyre, Malawi (1995) | Taha (1998) [218] | 98 | 808 | 12.1 | 9.9, 14.4 | RPR and TPHA | 11.4 | 9.2, 13.5 | 1 |
| | Dar es Salaam, Tanzania (1995-97) | Urassa (2001) [224] | 62 | 1,058 | 5.9 | 4.4, 7.3 | VDRL and TPHA | 5.0 | 3.7, 6.3 | 4 |
| | Addis Ababa, Ethiopia (1995-2001) | Tsegaye (2002) [225] | 135 | 4,731 | 2.9 | 2.4, 3.3 | RPR and TPHA | 1.9 | 1.5, 2.3 | 4 |
| | Blantyre, Malawi (1996) | Taha (1998) [218] | 92 | 829 | 11.1 | 9.0, 13.2 | RPR and TPHA | 10.3 | 8.2, 12.4 | 1 |
| | Kisumu, Kenya (1996-97) | Ayisi (2000) [226] | 65 | 2,844 | 2.3 | 1.7, 2.8 | VDRL only | 0.3 | 0.1, 0.5 | 1 |
| | Addis Ababa, Ethiopia (1997) | Kebede (2000) [227] | 12 | 410 | 2.9 | 1.3, 4.6 | VDRL and TPHA or VDRL and FTA-Abs | 2.0 | 0.6, 3.3 | 3 |
| | Nairobi, Kenya (1997) | Fonck (2000) [228] | 22 | 334 | 6.6 | 3.9, 9.3 | RPR only | 2.4 | 0.7, 4.0 | 2 |
| | Gutu, Zimbabwe (not reported) | Majoko (2003) [229] | 9 | 85 | 10.6 | 4.1, 17.1 | RPR only | 6.9 | 1.5, 12.3 | 23 |
| | Kisumu, Kenya (1996-99) | Ayisi (2003) [230] | 55 | 2,288 | 2.4 | 1.8, 3.0 | RPR only | 0.5* | 0.2, 0.8 | 1 |
| | Nairobi, Kenya (1997-98) | Fonck (2001) [231] | 928 | 27,377 | 3.4 | 3.2, 3.6 | RPR only | 0.5* | 0.4, 0.6 | 10 |
| | Khartoum, Sudan (1999) | Ortashi (2004) [232] | 11 | 151 | 7.3 | 3.1, 11.4 | RPR and TPHA | 6.4 | 1.5, 12.3 | 1 |
| 2000-2011 | Harare, Zimbabwe (Not reported) | Pham (2005) [233] | 74 | 2,969 | 2.5 | 1.9, 3.1 | RPR and TPHA | 1.5 | 1.1, 2.0 | 1 |
| | Moshi, Tanzania (1999-2004) | Msuya (2007) [234] | 39 | 3,046 | 1.3 | 0.9, 1.7 | RPR and MHA-TP | 0.3 | 0.1, 0.5 | 3 |
| | Gaborone, Botswana (2000-01) | Romoren (2006) [235] | 32 | 703 | 4.6 | 3.0, 6.1 | RPR and TPHA | 3.6 | 2.2, 5.0 | 13 |
| | Blantyre, Malawi (2000-04) | Kwiek (2008) [236] | 198 | 3,824 | 5.2 | 4.5, 5.9 | RPR and TPHA | 4.3 | 3.6, 4.9 | 1 |
| | Manhiça, Mozambique (2000) | Menendez (2010) [237] | 31 | 258 | 12.0 | 8.1, 16.0 | RPR and IgG ELISA | 11.24 | 7.39, 15.1 | 1 |
| | Harare, Zimbabwe (2002-03) | Kurewa (2010) [238] | 8 | 678 | 1.2 | 0.4, 2.0 | RPR and TPHA | 0.2 | -0.1, 0.5 | 3 |
| | Mwanza, Tanzania (2002) | Watson-Jones (2002) [120] | 106 | 1,809 | 5.9 | 4.8, 6.9 | RPR and TPHA | 5.0 | 3.0, 6.0 | 2 |
| | Harare, Zimbabwe (2002-04) | Mapingure (2010) [239] | 8 | 662 | 1.2 | 0.4, 2.0 | RPR and Determine Syphilis TP | 0.2 | -0.1, 0.5 | 1 |
| | Moshi, Tanzania (2002-04) | Mapingure (2010) [239] | 23 | 2654 | 0.9 | 0.5, 1.3 | RPR and Determine Syphilis TP | 0.5* | 0.2, 0.8 | 1 |
| | Multicentre, Tanzania (2003-04) | Swai (2006) [240] | 1,261 | 17,277 | 7.3 | 6.9, 7.7 | RPR only | 3.2 | 2.9, 3.4 | 57 |
| | Sofala, Mozambique (2003-04) | Montoya (2006) [241] | 32 | 381 | 8.4 | 5.6, 11.2 | RPR and TPHA | 7.5 | 4.9, 10.2 | 6 |
| | Rural Tanzania (2003-04) | Yahya-Malima (2008) [242] | 21 | 1,296 | 1.6 | 1.0, 2.5 | RPR and TPHA | 0.6 | 0.2, 1.1 | 6 |
| | Manhiça, Mozambique (2003-05) | Menendez (2008) [77] | 122 | 1,030 | 11.8 | 9.9, 13.8 | RPR only | 8.3 | 4.9, 10.2 | 1 |
| | Entebbe, Uganda (2004) | Tann (2006) [243] | 4 | 245 | 1.6 | 0.1, 3.2 | RPR and TPHA | 0.7 | -0.4, 1.7 | 1 |
| | Multicentre, Mozambique (2004) | Lujan (2008) [244] | 53 | 1,117 | 4.7 | 3.5, 6.0 | RPR and TPHA | 3.8 | 2.7, 5.0 | 2 |
| | Entebbe, Uganda (2003-05) | Woodburn (2009) [245] | 18 | 2,507 | 0.7 | 0.4, 1.0 | RPR and TPHA | 0.5* | 0.2, 0.8 | 1 |
| | Southern Malawi (2004-05) | Van Den Broek (2009) [108] | 163 | 2,297 | 7.1 | 6.1, 8.2 | VDRL only | 5.9 | 4.9, 6.8 | 4 |
| | North-west Somalia (2004) | Abdalla (2010) [246] | 21 | 1,559 | 1.3 | 0.8, 1.9 | RPR and TPHA | 0.4 | -0.1, 0.2 | 4 |
| | Gondar, Ethiopia (2005) | Mulu (2007) [247] | 5 | 480 | 1.0 | 0.1, 2.0 | RPR and TPHA | 4.5 | 3.9, 5.1 | 1 |

## Table 4.2 Syphilis point estimates: sub-Saharan Africa (continued)

| Site (year study conducted) | | Reference (year of publication) | No. women positive | No. women tested | Uncorrected prevalence (%) | 95% CI | Diagnostic method | Corrected prevalence (%) | 95% CI | No. ANC facilities |
|---|---|---|---|---|---|---|---|---|---|---|
| **West and Central Africa** | | | | | | | | | | |
| 1990-1999 | Kinshasa, Zaire (1990) | Vuylsteke (1993) [133] | 13 | 1,160 | 1.1 | 0.5, 1.7 | RPR and TPHA | 0.1 | -0.1, 0.3 | 4 |
| | Abidjan, Côte d'Ivoire (1992) | Diallo (1997) [248] | 6 | 545 | 1.1 | 0.2, 2.0 | RPR and TPHA | 0.1 | -0.2, 0.4 | 1 |
| | Cotonou, Benin (1993) | Rodier (1995) [249] | 5 | 205 | 2.4 | 0.3, 4.5 | VDRL and TPHA | 1.5 | -0.2, 3.1 | 1 |
| | Multicentre, Burkina Faso (1994) | Meda (1997) [250] | 23 | 645 | 3.6 | 2.1, 5.0 | RPR and TPHA | 2.6 | 1.4, 3.9 | 2 |
| | Libreville, Gabon (1994-95) | Bourgeois (1998) [251] | 19 | 646 | 2.9 | 1.6, 4.2 | RPR only | 0.5* | 0.05, 1.0 | 3 |
| | Yaounde, Cameroon (1994-96) | Mbopi Keou (1998) [210] | 696 | 4,100 | 17.0 | 15.8, 18.1 | TPHA only | 16.3 | 15.2, 17.4 | 1 |
| | Bangui, Central African Rep (1996) | Blankhart (1999) [252] | 30 | 451 | 6.7 | 4.4, 9.0 | RPR / TPHA or VDRL / TPHA | 5.8 | 3.6, 7.9 | 3 |
| | Bobo-Dioulasso, Burkina Faso (1995-98) | Sombie (2000) [253] | 26 | 10,980 | 0.2 | 0.2, 0.3 | RPR and TPHA | 0.5* | 0.4, 0.6 | 3 |
| | Enugu, Nigeria (1997-2001) | Ikeme (2006) [254] | 86 | 6,881 | 1.3 | 1.0, 1.5 | VDRL only | 0.5* | 0.4, 0.7 | 1 |
| 2000-2011 | Ilorin, Nigeria (2000) | Aboyeji (2003) [255] | 4 | 230 | 1.7 | 0.1, 3.4 | RPR and TPHA | 0.8 | -0.4, 1.9 | 1 |
| | Maiduguri, Nigeria (1999-2008) | Bukar (2009) [256] | 9 | 18,101 | 0.1 | 0.0, 0.1 | RPR and TPHA | 0.5* | 0.4, 0.6 | 1 |
| | Accra, Ghana (2000-01) | Apea-Kubi (2004) [257] | 21 | 294 | 7.1 | 4.2, 10.1 | RPR and TPHA | 6.3 | 3.5, 9.0 | 1 |
| | Jos, Nigeria (2002-03) | Sagay (2005) [258] | 7 | 2657 | 0.3 | 0.1, 0.5 | RPR and TPHA | 0.5* | 0.2, 0.8 | 1 |
| | Multicentre, Burkina Faso (2003) | Kirakoya (2010) [259] | 38 | 2133 | 1.8 | 1.2, 2.3 | VDRL and TPHA | 0.8 | 0.4, 1.2 | 98 |
| | Kinshasa, DRC (2004) | Kinoshita-Moleka (2008) [260] | 0 | 529 | 0.0 | 0.0, 0.0 | RPR and TPHA | 0.5* | -0.1, 1.1 | 2 |
| | Osogbo, Nigeria (2004-06) | Taiwo (2007) [261] | 15 | 505 | 3.0 | 1.5, 4.5 | RPR and TPHA | 2.0 | 0.8, 3.2 | 2 |

RPR = Rapid plasma reagin
VDRL = Venereal disease research laboratory
TPHA = *Treponema pallidum* haemagglutination
MHA-TP = Micro haemagglutination assay–*Treponema pallidum*
IgG = Immunoglobulin G
ELISA= Enzyme-linked immunosorbent assay
FTA-Abs = Fluorescent treponemal antibody absorption
* The adjusted point prevalence estimate was equal to zero with no variance and is not shown in corresponding figure

**Figure 4.2a Syphilis pooled estimates: East and Southern Africa**

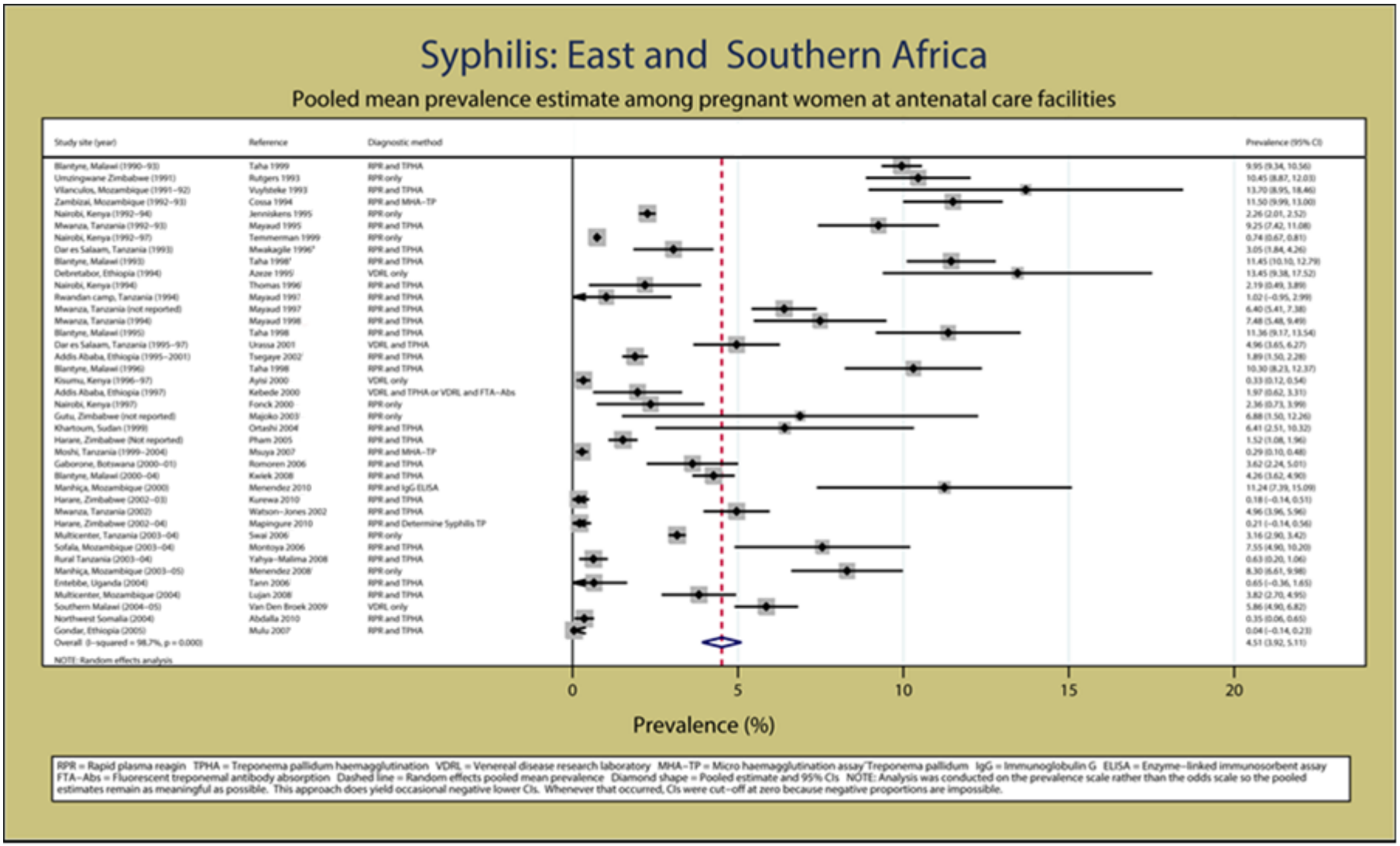
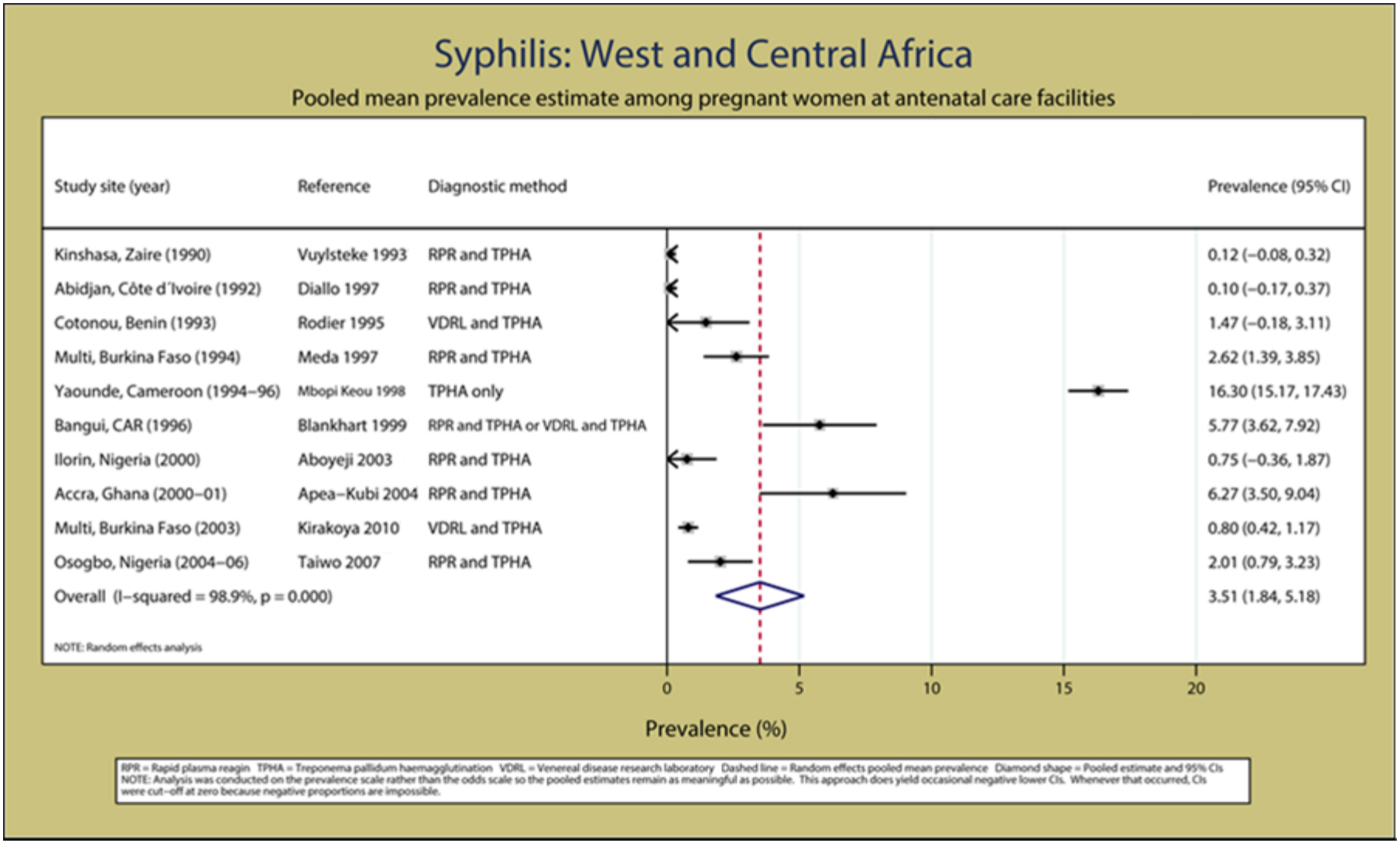


**Figure 4.2b Syphilis pooled estimates: West and Central Africa**



### *Neisseria gonorrhoeae* prevalence

Amongst women in East and Southern Africa, the pooled prevalence was 3.7% (95% CI: 2.8, 4.6) as shown in Figure 4.3a with the highest point estimate of 23.3% (95% CI: 16.4, 30.2) observed in Manhiça, Mozambique diagnosed by culture and gram stain[209] as presented in Table 4.3. The pooled prevalence of *N. gonorrhoeae* in West and Central Africa was 3.5% (95% CI: 1.8, 5.2) as shown in Figure 4.3b. The highest individual estimate, 4.6% (95% CI: 2.8, 6.3), came from a study in Abidjan, Côte d'Ivoire, where culture diagnostic methods were used[248] as presented in Table 4.3.

## Table 4.3 *Neisseria gonorrhoeae* point estimates: sub-Saharan Africa

| Site (year study conducted) | | Reference (year of publication) | No. women positive | No. women tested | Uncorrected prevalence (%) | 95% CI | Diagnostic method | Corrected prevalence (%) | 95% CI | No. ANC facilities |
|---|---|---|---|---|---|---|---|---|---|---|
| **East and Southern Africa** | | | | | | | | | | |
| 1990-1999 | Blantyre, Malawi (1990-93) | Taha (1999) [211] | 376 | 9104 | 4.1 | 3.7, 4.5 | Culture | 5.2 | 4.7, 5.6 | 1 |
| | Vilanculos, Mozambique (1991-92) | Vuylsteke (1993) [209] | 14 | 201 | 7.0 | 3.5, 10.5 | Culture | 8.7 | 4.8, 12.6 | 1 |
| | Mwanza, Tanzania (1992-93) | Mayaud (1995) [215] | 20 | 964 | 2.1 | 1.2, 3.0 | Culture | 2.6 | 1.6, 3.6 | 12 |
| | Dar es Salaam, Tanzania (1993) | Mwakagile (1996) [217] | 28 | 777 | 3.6 | 2.3, 4.9 | Culture | 4.5 | 3.1, 6.0 | 1 |
| | Blantyre, Malawi (1993) | Taha (1998) [218] | 54 | 2,161 | 2.5 | 1.8, 3.2 | Culture | 3.1 | 2.4, 3.9 | 1 |
| | Nairobi, Kenya (1994) | Thomas (1996) [220] | 7 | 286 | 2.5 | 0.7, 4.2 | Culture | 3.1 | 1.1, 5.1 | 1 |
| | Mwanza, Tanzania (1994) | Mayaud (1998) [223] | 15 | 660 | 2.3 | 1.1, 3.4 | Culture | 2.8 | 1.6, 4.1 | 1 |
| | Rwandan camp, Tanzania (1994) | Mayaud (1997) [221] | 3 | 100 | 3.0 | -0.3, 6.3 | Gram stain | 0.5* | -0.9, 1.9 | 1** |
| | Blantyre, Malawi (1995) | Taha (1998) [218] | 20 | 808 | 2.5 | 1.4, 3. | Culture | 3.1 | 1.9, 4.3 | 1 |
| | Nairobi, Kenya (1997) | Fonck (2000) [228] | 10 | 334 | 3.0 | 1.2, 4.8 | Culture | 3.7 | 1.7, 5.8 | 10 |
| | Khartoum, Sudan (1999) | Ortashi (2004) [232] | 3 | 151 | 2.0 | -0.2, 4.2 | Culture | 2.5 | 0.0, 5.0 | 1 |
| 2000-2011 | Gaborone, Botswana (2000-01) | Romoren (2004) [262] | 21 | 561 | 3.7 | 2.2, 5.3 | LCR cervical swab | 2.8 | 1.5, 4.2 | 13 |
| | Manhiça, Mozambique (2000) | Menendez (2010) [237] | 27 | 145 | 18.6 | 12.3, 25.0 | Culture and gram stain | 23.3 | 16.4, 30.2 | 1 |
| | Dar es Salaam, Tanzania (2001-03) | Aboud (2008) [263] | 1 | 428 | 0.2 | -0.2, 0.7 | Gram stain | 0.5* | -0.2, 1.2 | 1 |
| | Blantyre, Malawi (2001-03) | Aboud (2008) [263] | 2 | 474 | 0.4 | -0.2, 1.0 | Gram stain | 0.5* | -0.1, 1.1 | 1 |
| | Lilongwe, Malawi (2001-03) | Aboud (2008) [263] | 26 | 748 | 3.5 | 2.2, 4.8 | Gram stain | 0.5* | -0.01, 1.0 | 1 |
| | Lusaka, Zambia (2001-03) | Aboud (2008) [263] | 10 | 642 | 1.6 | 0.6, 2.5 | Gram stain | 0.5* | -0.05, 1.0 | 1 |
| | Moshi, Tanzania (1999-2004) | Msuya (2007) [234] | 17 | 3,046 | 0.6 | 0.3, 0.8 | Culture or gram stain | 0.5* | 0.3, 0.8 | 3 |
| | Entebbe, Uganda (2004) | Tann (2006) [243] | 10 | 233 | 4.3 | 1.7, 6.9 | PCR cervical swab | 1.4 | -0.1, 3.0 | 1 |
| | Multicentre, Mozambique (2004) | Lujan (2008) [244] | 21 | 835 | 2.5 | 1.5, 3.6 | PCR urine | 2.8 | 1.7, 3.9 | 2 |
| **West and Central Africa** | | | | | | | | | | |
| 1990-1999 | Kinshasa, Zaire (1990) | Vuylsteke (1993) [133] | 19 | 1,160 | 1.6 | 0.9, 2.3 | Culture | 2.1 | 1.2, 2.9 | 4 |
| | Ile-Ife, Nigeria (1990-91) | Okonofua (1995) [264] | 9 | 86 | 10.5 | 4.0, 17.0 | Gram stain | 0.5* | -1.0, 2.0 | 1 |
| | Abidjan, Côte d'Ivoire (1992) | Diallo (1997) [248] | 20 | 546 | 3.7 | 2.1, 5.2 | Culture | 4.6 | 2.8, 6.3 | 1 |
| | Praia, Cape Verde (1993) | Wessel (1998) [265] | 17 | 350 | 4.9 | 2.6, 7.1 | PCR cervical swab | 2.1 | 0.6, 3.6 | 1 |
| | Multi- Burkina Faso (1994) | Meda (1997) [250] | 23 | 645 | 3.6 | 2.1, 5.0 | Gram stain | 0.5* | -0.05, 1.0 | 2 |
| | Libreville, Gabon (1994-95) | Bourgeois (1998) [251] | 12 | 646 | 1.9 | 0.8, 2.9 | Culture or gram stain | 0.5* | -0.05, 1.0 | 3 |
| | Bangui, Cent African Rep (1996) | Blankhart (1999) [252] | 14 | 451 | 3.1 | 1.5, 4.7 | Culture | 3.9 | 2.1, 5.7 | 3 |
| | Abidjan, Côte d'Ivoire (1996-97) | Faye-Kette (2000) [266] | 9 | 551 | 1.6 | 0.6, 2.7 | Gram stain | 0.5* | -0.1, 1.1 | 1 |
| 2000-2011 | Ilorin, Nigeria (2000) | Aboyeji (2003) [255] | 3 | 230 | 1.3 | -0.2, 2.8 | Culture | 1.6 | -0.0, 3.3 | 1 |
| | Accra, Ghana (2000-01) | Apea-Kubi (2004) [257] | 1 | 261 | 0.4 | -0.4, 1.1 | PCR cervical swab | 0.5* | -0.4, 1.4 | 1 |
| | Kinshasa, Dem Rep Congo (2004) | Kinoshita-Moleka (2008) [260] | 2 | 521 | 0.4 | -0.2, 0.9 | PCR cervical swab | 0.5* | -0.1, 1.1 | 2 |

PCR = Polymerase chain reaction

LCR = Ligase chain reaction

* The adjusted point prevalence estimate was equal to zero with no variance and is not shown in corresponding figure

** Refugee camp

**Figure 4.3a *Neisseria gonorrhoeae* pooled estimates: East and Southern Africa**

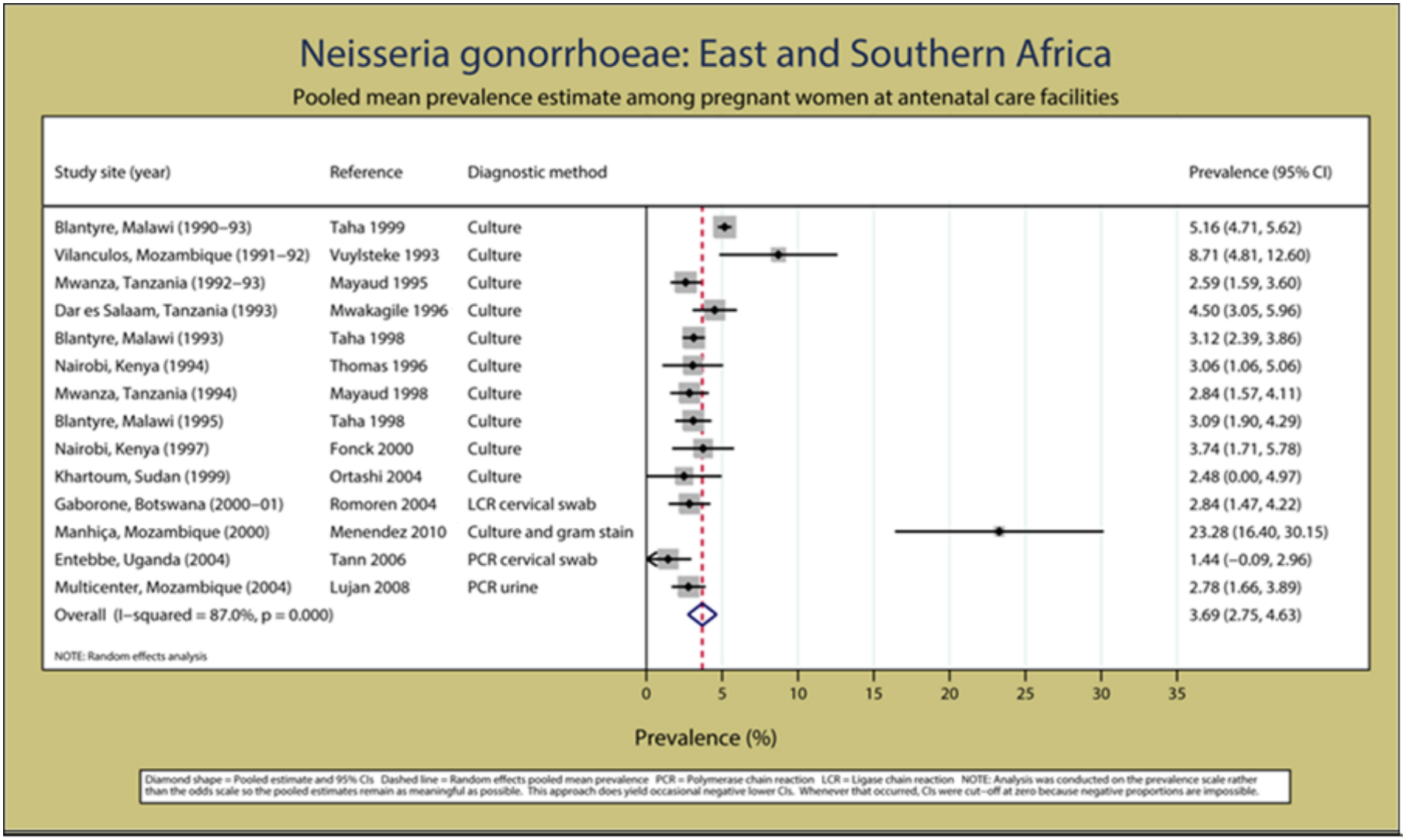
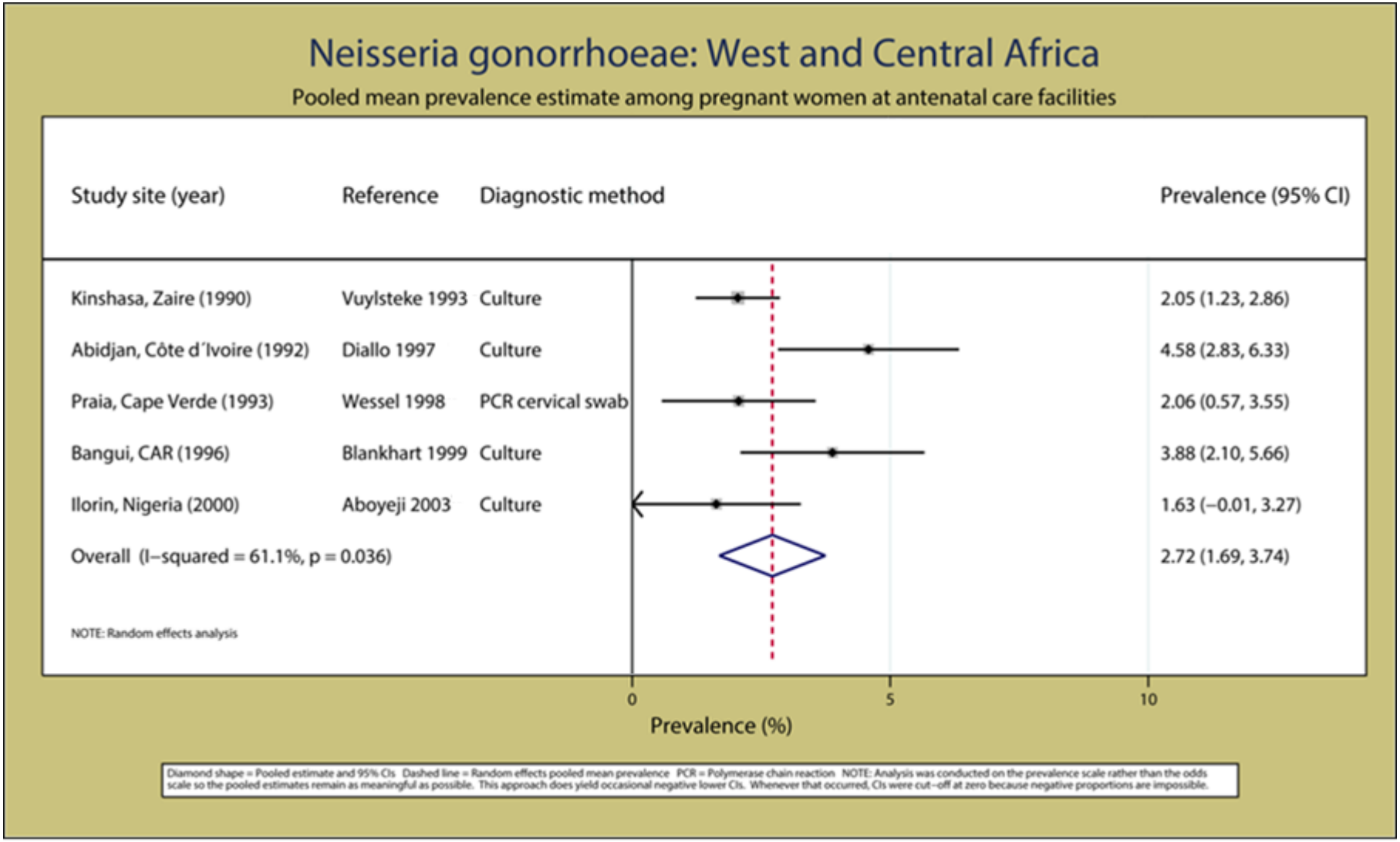


**Figure 4.3b *Neisseria gonorrhoeae* pooled estimates: West and Central Africa**



### *Chlamydia trachomatis* prevalence

In East and Southern Africa, the pooled prevalence of *C. trachomatis* infection was 6.9% (95% CI: 5.1, 8.6) as shown in Figure 4.4a. Khartoum, Sudan, reported the highest prevalence estimate of 23.2% (95% CI: 16.5, 29.9) as detected by an enzyme immunoassay (EIA)[232] as presented in Table 4.4. In West and Central Africa, the pooled mean prevalence was 6.1% (95% CI: 4.0, 8.3) as shown in Figure 4.4b. The highest prevalence, 16.4% (95% CI: 12.6, 20.1) based on culture or EIA, was observed in Pikine, Senegal[267] as presented in Table 4.4.

## Table 4.4 *Chlamydia trachomatis* point estimates: sub-Saharan Africa

| Site (year study conducted) | | Reference (year of publication) | No. women positive | No. women tested | Uncorrected prevalence (%) | 95% CI | Diagnostic method | Corrected prevalence (%) | 95% CI | No. ANC facilities |
|---|---|---|---|---|---|---|---|---|---|---|
| **East and Southern Africa** | | | | | | | | | | |
| **1990-1999** | Vilanculos, Mozambique (1991-92) | Vuylsteke (1993) [209] | 11 | 141 | 7.8 | 3.4, 12.2 | Culture cervical swab | 0.5* | -0.7, 1.7 | 1 |
| | Mwanza, Tanzania (1992-93) | Mayaud (1995) [215] | 64 | 964 | 6.6 | 5.1, 8.2 | EIA | 6.0 | 4.5, 7.5 | 12 |
| | Nairobi, Kenya (1994) | Thomas (1996) [220] | 25 | 286 | 8.7 | 5.5, 12.0 | EIA | 8.8 | 5.5, 12.0 | 1 |
| | Mwanza, Tanzania (1994) | Mayaud (1998) [223] | 39 | 660 | 5.9 | 4.1, 7.7 | EIA | 5.1 | 3.4, 6.8 | 1 |
| | Nairobi, Kenya (1997) | Fonck (2000) [228] | 36 | 334 | 10.8 | 7.5, 14.1 | PCR cervical swab | 10.4 | 7.2, 13.7 | 2 |
| | Khartoum, Sudan (1999) | Ortashi (2004) [232] | 30 | 151 | 19.9 | 13.5, 26.2 | EIA | 23.2 | 16.5, 29.9 | 1 |
| **2000-2011** | Gaborone, Botswana (2000-01) | Romoren (2004) [262] | 42 | 557 | 7.5 | 5.4, 9.7 | LCR cervical swab | 7.6 | 5.3, 9.7 | 13 |
| | Manhiça, Mozambique (2000) | Menendez (2010) [237] | 15 | 151 | 9.9 | 5.2, 14.7 | DNA ID-assay cervical swab | 9.1 | 4.5, 13.7 | 1 |
| | Lusaka, Zambia (2001-03) | Aboud (2008) [263] | 39 | 642 | 6.1 | 4.2, 7.9 | EIA | 5.3 | 3.6, 7.0 | 1 |
| | Dar es Salaam, Tanzania (2001-03) | Aboud (2008) [263] | 12 | 343 | 3.5 | 1.6, 5.4 | EIA | 2.0 | 0.5, 3.4 | 1 |
| | Blantyre, Malawi (2001-03) | Aboud (2008) [263] | 4 | 474 | 0.8 | 0.0, 1.7 | EIA | 0.5* | -0.1, 1.2 | 1 |
| | Lilongwe, Malawi (2001-03) | Aboud (2008) [263] | 2 | 748 | 0.3 | -0.1, 0.6 | EIA | 0.5* | 0.01, 1.0 | 1 |
| | Entebbe, Uganda (2004) | Tann (2006) [243] | 14 | 236 | 5.9 | 2.9, 9.0 | PCR cervical swab | 5.5 | 2.6, 8.4 | 1 |
| | Multicentre, Mozambique (2004) | Lujan (2008) [244] | 34 | 835 | 4.1 | 2.7, 5.4 | PCR urine | 4.3 | 2.9, 5.7 | 2 |
| **West and Central Africa** | | | | | | | | | | |
| **1990-1999** | Pikine, Senegal (1990) | Van Dyck (1992) [267] | 55 | 377 | 14.6 | 11.0, 18.2 | Culture or EIA | 16.4 | 12.6, 20.1 | 1 |
| | Kinshasa, Zaire (1990) | Vuylsteke (1993) [133] | 60 | 1,160 | 5.2 | 3.9, 6.5 | EIA | 4.1 | 3.0, 5.3 | 4 |
| | Abidjan, Côte d'Ivoire (1992) | Diallo (1997) [248] | 25 | 452 | 5.5 | 3.4, 7.6 | EIA | 4.6 | 2.7, 6.5 | 1 |
| | Praia, Cape Verde (1993) | Wessel (1998) [265] | 46 | 350 | 13.1 | 9.6, 16.7 | PCR cervical swab | 12.9 | 9.3, 16.3 | 1 |
| | Multicentre, Burkina Faso (1994) | Meda (1997) [250] | 20 | 645 | 3.1 | 1.8, 4.4 | EIA | 1.4 | 0.5, 2.4 | 2 |
| | Libreville, Gabon (1994-95) | Bourgeois (1998) [251] | 64 | 646 | 9.9 | 7.6, 12.2 | EIA | 10.3 | 7.9, 12.6 | 3 |
| | Bangui, CAR (1996) | Blankhart (1999) [252] | 28 | 451 | 6.2 | 4.0, 8.4 | Indirect immunofluorescence | 4.3 | 2.5, 6.2 | 3 |
| | Abidjan, Côte d'Ivoire (1996-97) | Faye-Kette (2000) [266] | 41 | 551 | 7.4 | 5.3, 9.6 | EIA | 7.1 | 4.9, 9.2 | 1 |
| **2000-2011** | Accra, Ghana (2000-01) | Apea-Kubi (2004) [257] | 9 | 261 | 3.5 | 1.2, 5.7 | PCR cervical swab | 3.0 | 0.9, 5.1 | 1 |
| | Kinshasa, DRC (2004) | Kinoshita-Moleka (2008) [260] | 9 | 521 | 1.7 | 0.6, 2.9 | PCR cervical swab | 6.1 | 4.0, 8.3 | 2 |

PCR = Polymerase chain reaction
LCR = Ligase chain reaction
EIA = Enzyme immunoassay
* The adjusted point prevalence estimate was equal to zero with no variance and is not shown in corresponding figure

**Figure 4.4a *Chlamydia trachomatis* pooled estimates: East and Southern Africa**

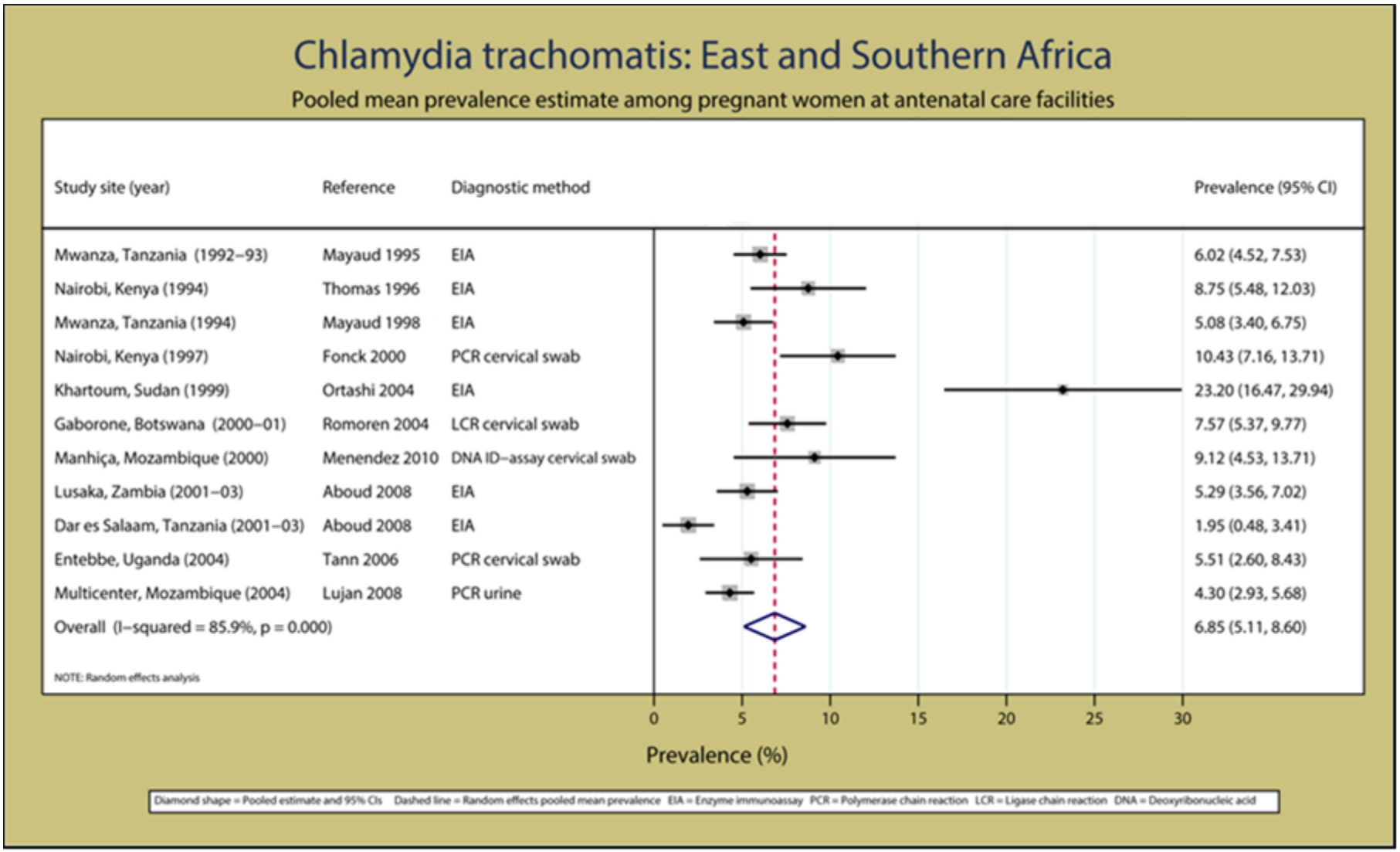
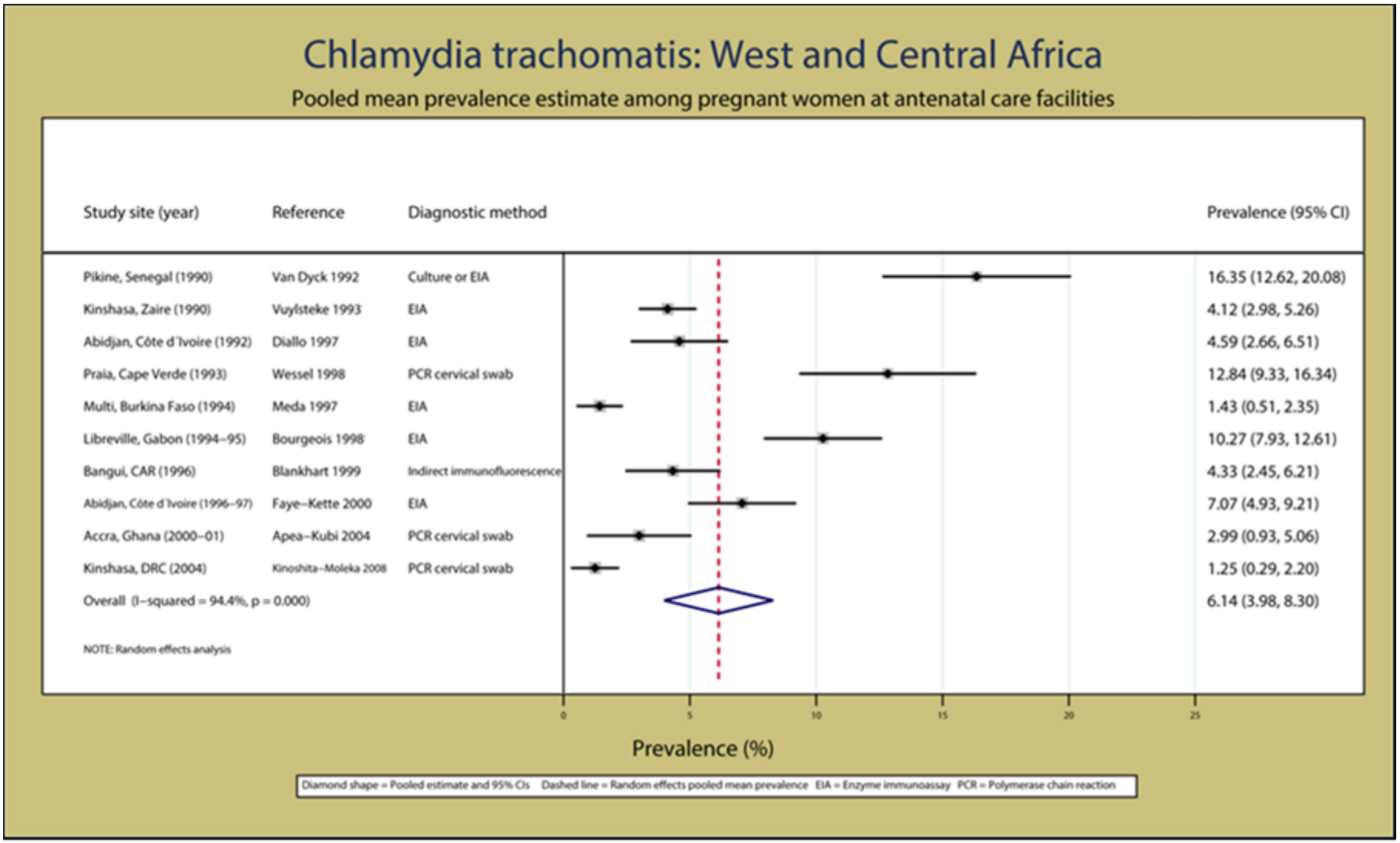


**Figure 4.4b *Chlamydia trachomatis* pooled estimates: West and Central Africa**



### *Trichomonas vaginalis* prevalence

The pooled prevalence of *T. vaginalis* infection was 29.1% (95% CI: 20.9, 37.2) amongst women in East and Southern Africa as shown in Figure 4.5a, compared to 17.8% (95% CI: 12.4, 23.1) in West and Central Africa illustrated in Figure 4.5b. Table 4.5 shows the highest point estimate in East and Southern Africa was 51.7% (95% CI: 41.9, 61.5), reported in Blantyre, Malawi[211] as diagnosed by wet-mount microscopy, whereas the highest prevalence estimate in West and Central Africa, 52.0% (95% CI: 47.6, 56.4), was in Jos, Nigeria, where the same diagnostic method was used.[268]

## Table 4.5 *Trichomonas vaginalis* point estimates: sub-Saharan Africa

| Site (year study conducted) | | Reference (year of publication) | No. women positive | No. women tested | Uncorrected prevalence (%) | 95% CI | Diagnostic method | Corrected prevalence (%) | 95% CI | No. ANC facilities |
|---|---|---|---|---|---|---|---|---|---|---|
| **East and Southern Africa** | | | | | | | | | | |
| 1990-1999 | Blantyre, Malawi (1990-93) | Taha (1999) [211] | 2,838 | 9,137 | 31.1 | 30.2, 32.1 | Wet-mount microscopy | 51.6 | 50.5, 52.6 | 1 |
| | Vilanculos, Mozambique (1991-92) | Vuylsteke (1993) [209] | 46 | 201 | 22.9 | 17.1, 28.7 | Wet-mount microscopy | 38.1 | 31.4, 44.9 | 1 |
| | Mwanza, Tanzania (1992-93) | Mayaud (1995) [215] | 264 | 964 | 27.4 | 24.6, 30.2 | Wet-mount microscopy | 45.6 | 42.5, 48.8 | 12 |
| | Dar es Salaam, Tanzania (1993) | Mwakagile (1996) [217] | 176 | 777 | 22.7 | 19.7, 25.6 | Wet-mount microscopy | 37.8 | 34.3, 41.2 | 1 |
| | Blantyre, Malawi (1993) | Taha (1998) [218] | 62 | 2,161 | 2.9 | 2.2, 3.6 | Wet-mount microscopy | 4.8 | 3.8, 5.7 | 1 |
| | Nairobi, Kenya (1994) | Thomas (1996) [220] | 57 | 286 | 19.9 | 15.3, 24.6 | Wet-mount microscopy | 33.2 | 27.8, 38.7 | 1 |
| | Mwanza, Tanzania (1994) | Mayaud (1998) [223] | 108 | 660 | 16.4 | 13.5, 19.2 | Wet-mount microscopy | 27.3 | 23.9, 30.7 | 1 |
| | Rwandan camp, Tanzania (1994) | Mayaud (1997) [221] | 31 | 100 | 31.0 | 21.9, 40.1 | Wet-mount microscopy | 51.7 | 41.9, 61.5 | 1* |
| | Mwanza, Tanzania (not reported) | Mayaud (1997) [222] | 627 | 2,282 | 27.5 | 25.6, 29.3 | Wet-mount microscopy | 45.8 | 43.8, 47.8 | 12 |
| | Blantyre, Malawi (1995) | Taha (1998) [218] | 19 | 808 | 2.4 | 1.3, 3.4 | Wet-mount microscopy | 3.9 | 2.6, 5.3 | 1 |
| | Nairobi, Kenya (1997) | Fonck (2000) [228] | 86 | 334 | 25.8 | 21.1, 30.4 | Wet-mount microscopy | 42.9 | 37.6, 48.2 | 2 |
| 2000-2011 | Khartoum, Sudan (1999) | Ortashi (2004) [232] | 11 | 151 | 7.3 | 3.1, 11.4 | Wet-mount microscopy | 12.1 | 6.9, 17.4 | 1 |
| | Moshi, Tanzania (1999-2004) | Msuya (2007) [234] | 207 | 2,917 | 7.1 | 6.2, 8.0 | Wet-mount microscopy | 11.8 | 10.7, 13.0 | 3 |
| | Gaborone, Botswana (2000-01) | Romoren (2007) [269] | 132 | 703 | 18.8 | 15.9, 21.7 | Wet-mount microscopy | 31.3 | 27.9, 34.7 | 13 |
| | Manhiça, Mozambique (2000) | Menendez (2010) [237] | 78 | 254 | 30.7 | 25.0, 36.4 | Wet-mount microscopy | 51.2 | 45.0, 57.3 | 1 |
| | Lilongwe, Malawi (2001-03) | Aboud (2008) [263] | 180 | 748 | 24.1 | 21.0, 27.1 | Wet-mount microscopy | 40.1 | 36.6, 43.6 | 1 |
| | Lusaka, Zambia (2001-03) | Aboud (2008) [263] | 134 | 642 | 20.9 | 17.7, 24.0 | Wet-mount microscopy | 34.8 | 31.1, 38.5 | 1 |
| | Blantyre, Malawi (2001-03) | Aboud (2008) [263] | 98 | 474 | 20.7 | 17.0, 24.3 | Wet-mount microscopy | 34.5 | 30.2, 38.7 | 1 |
| | Dar es Salaam, Tanzania (2001-03) | Aboud (2008) [263] | 18 | 428 | 4.2 | 2.3, 6.1 | Wet-mount microscopy | 7.0 | 4.6, 9.4 | 1 |
| | Moshi, Tanzania (2002-04) | Mapingure (2010) [239] | 127 | 2,555 | 5.0 | 4.2, 5.9 | Wet-mount microscopy | 8.3 | 7.2, 9.4 | 1 |
| | Harare, Zimbabwe (2002-04) | Mapingure (2010) [239] | 80 | 680 | 11.8 | 9.4, 14.2 | Wet-mount microscopy | 19.6 | 16.6, 22.6 | 1 |
| | Harare, Zimbabwe (2002-03) | Kurewa (2010) [238] | 80 | 678 | 11.8 | 9.4, 14.2 | Wet-mount microscopy | 19.7 | 16.7, 22.7 | 3 |
| | Entebbe, Uganda (2004) | Tann (2006) [243] | 43 | 249 | 17.3 | 12.6, 22.0 | In-pouch culture | 18.0 | 13.2, 22.8 | 1 |
| **West and Central Africa** | | | | | | | | | | |
| 1990-1999 | Jos, Nigeria (not reported) | Ogbonna (1991) [268] | 156 | 500 | 31.2 | 27.1, 35.3 | Wet-mount microscopy | 52.0 | 47.6, 56.4 | 2 |
| | Kinshasa, Zaire (1990) | Vuylsteke (1993) [133] | 213 | 1,160 | 18.4 | 16.1, 20.6 | Wet-mount microscopy | 30.6 | 28.0, 33.3 | 4 |
| | Ile-Ife, Nigeria (1990-91) | Okonofua (1995) [264] | 2 | 86 | 2..3 | -0.9, 5.5 | Wet-mount microscopy | 3.9 | -0.2, 8.0 | 1 |
| | Abidjan, Côte d'Ivoire (1992) | Diallo (1997) [248] | 72 | 546 | 13.2 | 10.3, 16.0 | Wet-mount microscopy | 22.0 | 18.5, 25.5 | 1 |
| | Multicentre, Burkina Faso (1994) | Meda (1997) [250] | 90 | 645 | 14.0 | 11.3, 16.6 | Wet-mount microscopy | 23.3 | 20.0, 26.5 | 2 |
| | Libreville, Gabon (1994-95) | Bourgeois (1998) [251] | 69 | 646 | 10.7 | 8.3, 13.1 | Wet-mount microscopy | 17.8 | 14.9, 20.8 | 3 |
| | Bangui, Central African Rep (1996) | Blankhart (1999) [252] | 45 | 451 | 10.0 | 7.2, 12.7 | Wet-mount microscopy | 16.6 | 13.2, 20.1 | 3 |
| | Abidjan, Côte d'Ivoire (1996-97) | Faye-Kette (2000) [266] | 74 | 551 | 13.4 | 10.6, 16.3 | Wet-mount microscopy | 22.4 | 18.9, 25.9 | 1 |
| 2000-2011 | Ilorin, Nigeria (2000) | Aboyeji (2003) [255] | 11 | 230 | 4.8 | 2.0, 7.5 | Wet-mount microscopy | 8.0 | 4.5, 11.5 | 1 |
| | Jos, Nigeria (2002-03) | Sagay (2005) [258] | 41 | 2,657 | 1.5 | 1.0, 2.0 | Wet-mount microscopy | 2.6 | 2.0, 3.2 | 1 |
| | North-east Nigeria (2003-04) | Nwosu (2007) [270] | 17 | 201 | 8.4 | 4.6, 12.3 | Wet-mount microscopy | 14.1 | 9.3, 18.9 | 1 |
| | Multicentre, Burkina Faso (2003) | Kirakoya (2008) [271] | 32 | 2,133 | 1.5 | 1.0, 2.0 | In-pouch culture | 1.6 | 1.0, 2.1 | 4 |

* Refugee camp

**Figure 4.5a *Trichomonas vaginalis* pooled estimates: East and Southern Africa**

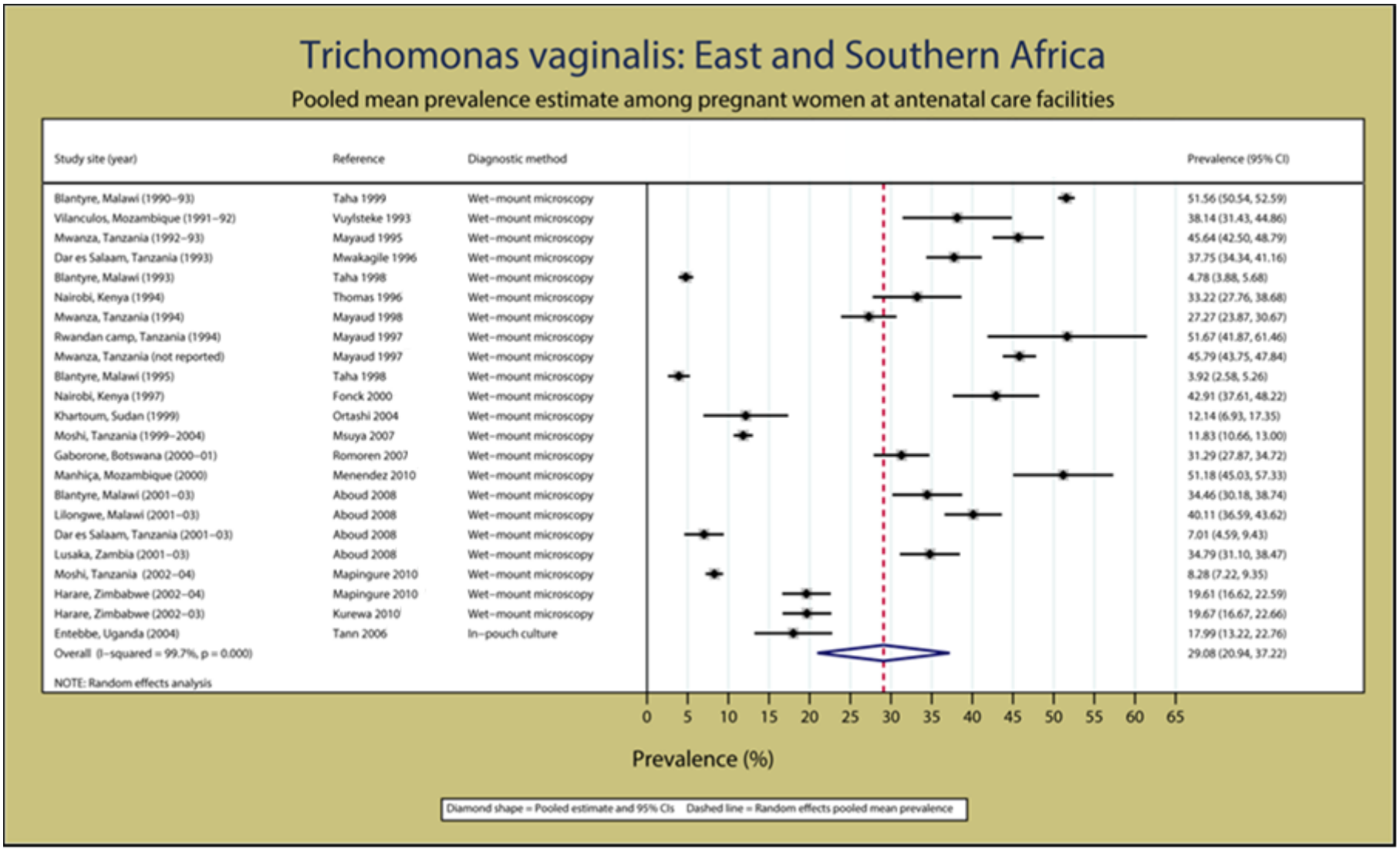
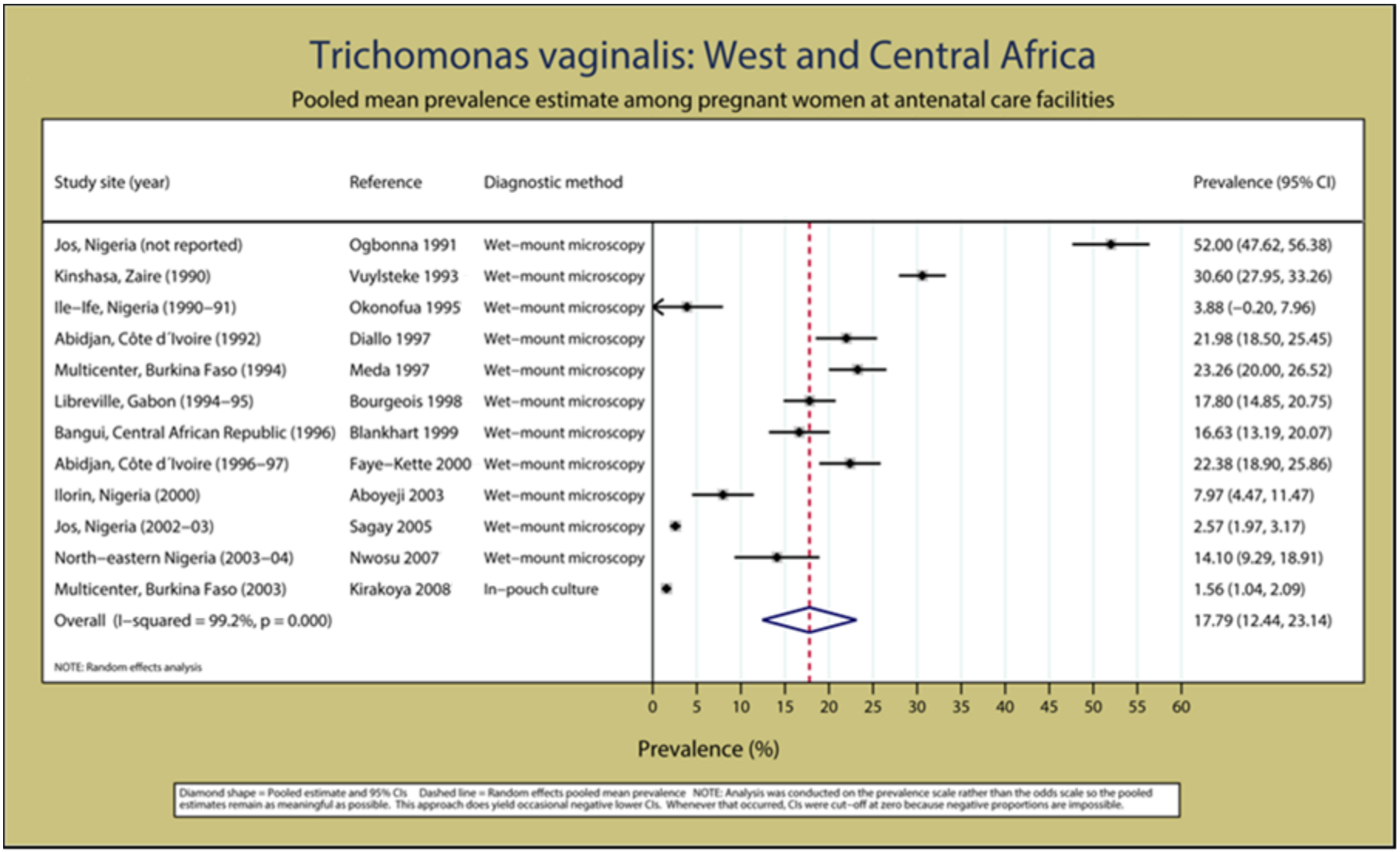


**Figure 5.5b *Trichomonas vaginalis* pooled estimates: West and Central Africa**



**Bacterial vaginosis prevalence**

The prevalence of bacterial vaginosis was higher than any other STI/RTI in both sub-regions. In East and Southern Africa, the pooled prevalence was 50.8% (95% CI: 43.4, 58.4) as shown in Figure 4.6a. The highest point estimate was 85.5% (95% CI: 82.8, 88.1) from Blantyre, Malawi where Amsel's criteria were used for diagnosis[211] as presented in Table 4.6. In West and Central Africa the pooled prevalence estimate was 37.6% (95% CI: 18.0, 57.2) as shown in Figure 4.6b with the highest point estimate based on clue cell count being 74.5% (95% CI: 70.0, 79.1) in Praia, Cape Verde[265] as presented in Table 4.6.

## Table 4.6 Bacterial vaginosis point estimates: sub-Saharan Africa

| Site (year study conducted) | | Reference (year of publication) | No. women positive | No. women tested | Uncorrected prevalence (%) | 95% CI | Diagnostic method | Corrected prevalence (%) | 95% CI | No. ANC facilities |
|---|---|---|---|---|---|---|---|---|---|---|
| **East and Southern Africa** | | | | | | | | | | |
| 1990-1999 | Blantyre, Malawi (1990) | Taha (1999) [211] | 640 | 2077 | 30.8 | 28.8, 32.8 | Amsel | 58.8 | 56.7, 60.9 | 1 |
| | Blantyre, Malawi (1993) | Taha (1999) [211] | 290 | 661 | 43.9 | 40.1, 47.7 | Amsel | 85.5 | 82.8, 88.1 | 1 |
| | Nairobi, Kenya (1994) | Thomas (1996) [220] | 59 | 286 | 20.6 | 15.9, 25.3 | Clue cell count | 47.7 | 41.9, 53.4 | 1 |
| | Rwandan camp, Tanzania (1994) | Mayaud (1997) [221] | 16 | 100 | 16.0 | 8.8, 23.2 | Clue cell count | 35.1 | 25.8, 44.5 | 1* |
| | Mwanza, Tanzania (1994) | Mayaud (1998) [223] | 159 | 660 | 24.1 | 20.8, 27.4 | Clue cell count | 57.0 | 53.2, 60.8 | 1 |
| | Multicentre, Zimbabwe (1999-2001) | Tolosa (2006) [272] | 51 | 209 | 24.4 | 18.6, 30.2 | Nugent | 23.5 | 17.8, 29.3 | 1 |
| | Moshi, Tanzania (1999-2004) | Msuya (2007) [234] | 661 | 3,046 | 21.7 | 20.2, 23.2 | Amsel | 40.2 | 38.5, 42.0 | 3 |
| 2000-2011 | Gaborone, Botswana (2000-01) | Romoren (2007) [269] | 264 | 703 | 37.6 | 34.0, 41.1 | Nugent | 39.5 | 35.9, 43.1 | 13 |
| | Lilongwe, Malawi (2001-03) | Aboud (2008) [263] | 329 | 748 | 44.0 | 40.4, 47.5 | Nugent | 47.3 | 43.7, 50.8 | 1 |
| | Blantyre, Malawi (2001-03) | Aboud (2008) [263] | 239 | 474 | 50.4 | 45.9, 54.9 | Nugent | 55.1 | 50.6, 59.6 | 1 |
| | Lusaka, Zambia (2001-03) | Aboud (2008) [263] | 251 | 642 | 39.1 | 35.3, 42.9 | Nugent | 41.3 | 37.5, 45.14 | 1 |
| | Dar es Salaam, Tanzania (2001-03) | Aboud (2008) [263] | 255 | 428 | 59.6 | 54.9, 64.2 | Nugent | 66.2 | 61.7, 70.7 | 1 |
| | Harare, Zimbabwe (2002-04) | Mapingure (2010) [239] | 195 | 598 | 32.6 | 28.8, 36.4 | Amsel | 62.5 | 58.6, 66.4 | 1 |
| | Moshi, Tanzania (2002-04) | Mapingure (2010) [239] | 533 | 2555 | 20.9 | 19.3, 22.5 | Amsel | 38.5 | 36.6, 40.4 | 1 |
| | Harare, Zimbabwe (2002-03) | Kurewa (2010) [238] | 221 | 678 | 32.6 | 29.1, 36.1 | Amsel | 62.4 | 58.8, 66.1 | 3 |
| | Entebbe, Uganda (2004) | Tann (2006) [243] | 117 | 247 | 47.4 | 41.1, 53.6 | Nugent | 51.4 | 45,1, 58.0 | 1 |
| **West and Central Africa** | | | | | | | | | | |
| 1999-1999 | Praia, Cape Verde (1993) | Wessel (1998) [265] | 107 | 350 | 30.6 | 25.7, 35.4 | Clue cell count | 74.5 | 70.0, 79.1 | 1 |
| | Multicentre, Burkina Faso (1994) | Meda (1997) [250] | 83 | 645 | 12.9 | 10.3, 15.5 | Amsel | 22.2 | 19.0, 25.4 | 2 |
| | Libreville, Gabon (1994-95) | Bourgeois (1998) [251] | 148 | 646 | 22.9 | 19.7, 26.2 | Nugent | 21.8 | 18.5, 24.9 | 3 |
| | Bangui, Central African Rep (1996) | Blankhart (1999) [252] | 132 | 453 | 29.1 | 25.0, 33.3 | Clue cell count | 70.7 | 66.5, 74.8 | 3 |
| | Abidjan, Côte d'Ivoire (1996-97) | Faye-Kette (2000) [266] | 170 | 551 | 30.9 | 27.0, 34.7 | Spiegel | 41.1 | 37.0, 45.3 | 1 |
| 2000-2011 | Jos, Nigeria (2002-03) | Sagay (2005) [258] | 466 | 2657 | 17.5 | 16.1, 18.9 | Amsel | 31.7 | 29.9, 33.5 | 1 |
| | Multicentre, Burkina Faso (2003) | Kirakoya-Moleka (2008) [271] | 138 | 2,133 | 6.5 | 5.4, 7.5 | Nugent | 1.8 | 1.2, 2.3 | 2 |

* Refugee camp

**Figure 4.6a Bacterial vaginosis pooled estimates: East and Southern Africa**

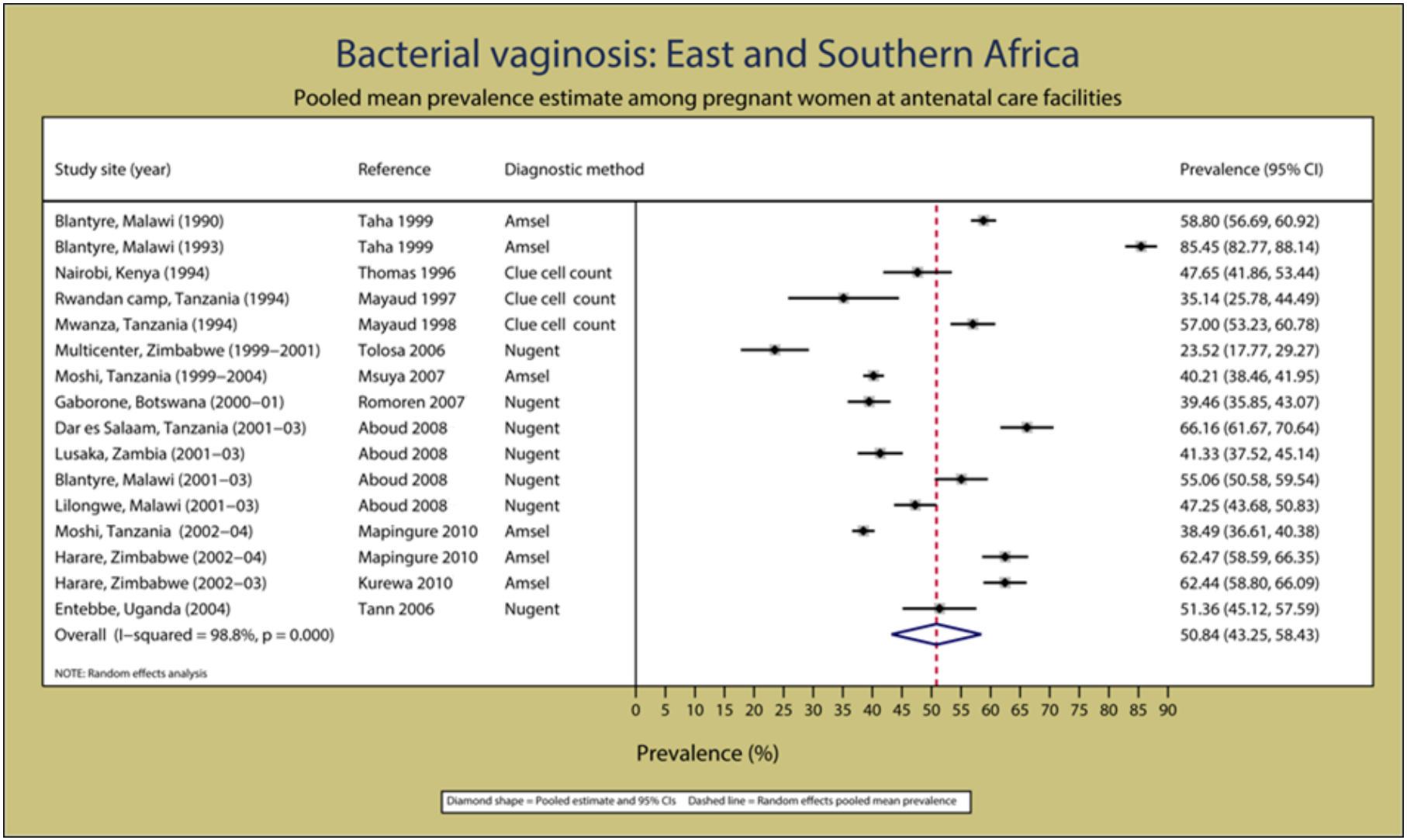
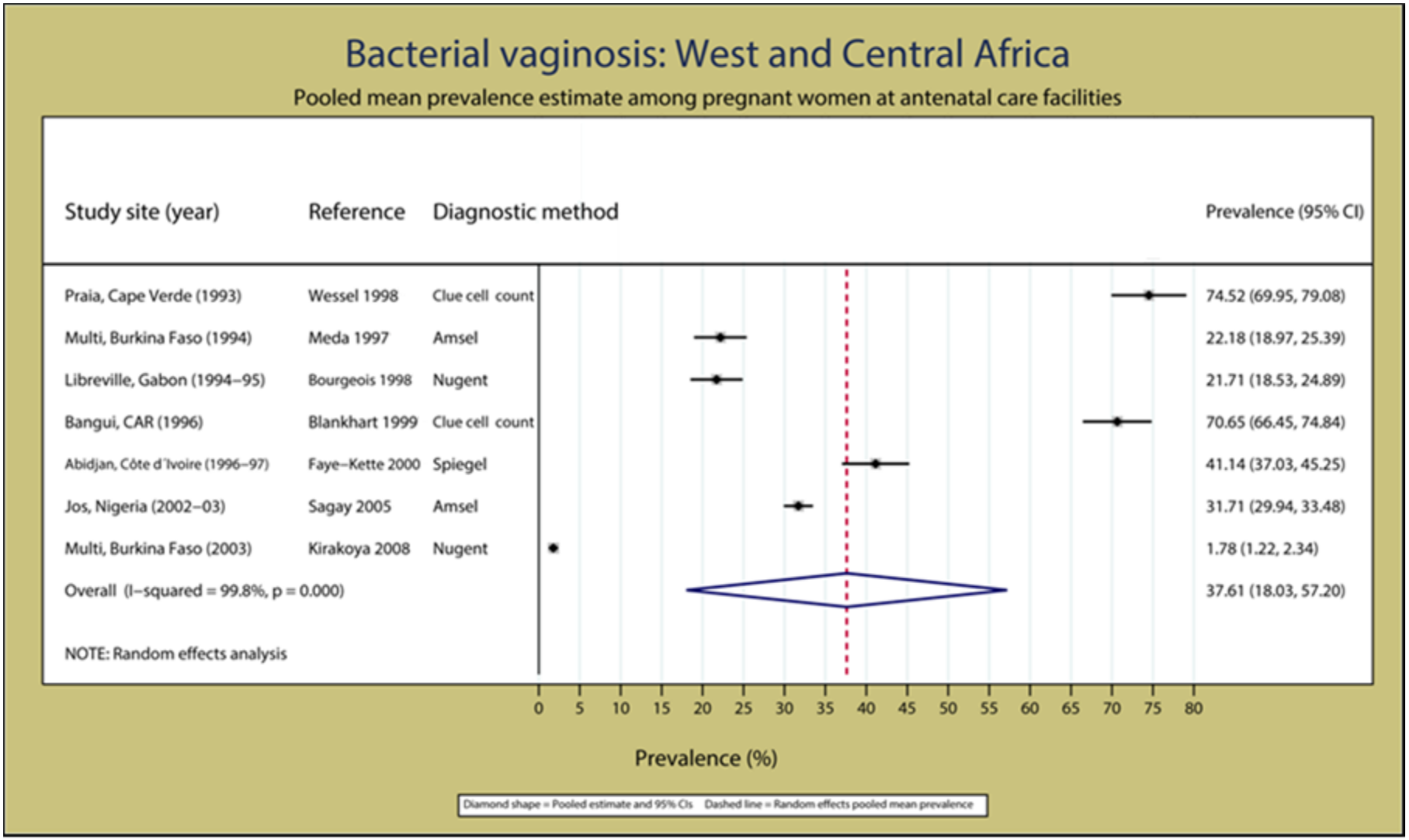


**Figure 4.6b Bacterial vaginosis pooled estimates: West and Central Africa**



**Peripheral malaria prevalence**

The pooled prevalence of peripheral malaria in East and Southern Africa was 32.0% (95% CI: 25.9, 38.0) as shown in Figure 4.7a. The highest prevalence in the sub-region, 87.9% (95% CI: 85.7, 90.1), was reported from Hoima, Uganda, where microscopy was used[273] as presented in Table 4.7. In West and Central Africa, the pooled prevalence of peripheral parasitaemia was 38.2% (95% CI: 32.3, 44.1) as shown in Figure 4.7b. The highest point estimate was 94.9% (95% CI: 88.0, 102.0) in Ngali II, Nigeria, where PCR methods were used[274] as presented in Table 4.7.

## Table 4.7 Peripheral malaria point estimates: sub-Saharan Africa

| Site (year study conducted) | | Reference (year of publication) | No. women positive | No. women tested | Uncorrected prevalence (%) | 95% CI | Diagnostic method | Corrected prevalence (%) | 95% CI | No. ANC facilities |
|---|---|---|---|---|---|---|---|---|---|---|
| **East and Southern Africa** | | | | | | | | | | |
| 1990-1999 | Kilifi, Kenya (1993) | Shulman (1996) [29] | 65 | 275 | 23.6 | 18.6, 28.7 | Giemsa stain microscopy | 28.7 | 23.3, 34.0 | 1 |
| | Chikwawa, Malawi (1993-94) | Verhoeff (1998) [38] | 109 | 575 | 18.9 | 15.7, 22.1 | Giemsa stain microscopy | 21.5 | 18.1, 24.8 | 1 |
| | Chikwawa, Malawi (1993-95) | Verhoeff (1999) [164] | 743 | 3,913 | 19.0 | 17.8, 20.2 | Giemsa stain microscopy | 21.5 | 20.2, 22.8 | 1 |
| | Kisumu, Kenya (1994-96) | Parise (1998) [74] | 330 | 736 | 44.8 | 41.2, 48.4 | Giemsa stain microscopy | 61.3 | 57.8, 64.8 | 2 |
| | Hoima, Uganda (1996-98) | Ndyomugyenyi (1999) [273] | 530 | 853 | 62.1 | 58.9, 65.4 | Giemsa stain microscopy | 87.9 | 85.7, 90.1 | 1 |
| | Kisumu, Kenya (1996-97) | Ayisi (2000) [226] | 583 | 2,844 | 20.5 | 19.0, 22.0 | Giemsa stain microscopy | 23.9 | 22.3, 25.4 | 1 |
| | Kisumu, Kenya (1996-98) | Van Eijk (2001) [275] | 953 | 4,608 | 20.7 | 19.5, 21.9 | Giemsa stain microscopy | 24.1 | 22.9, 25.4 | 1 |
| | Kisumu, Kenya (1996-97) | Van Eijk (2003) [276] | 380 | 2,502 | 15.2 | 13.8, 16.6 | Giemsa stain microscopy | 15.7 | 14.3, 17.1 | 1 |
| | Kisumu, Kenya (1996-99) | Van Eijk (2002) [277] | 1,105 | 5,093 | 21.7 | 20.6, 22.8 | Giemsa stain microscopy | 25.7 | 24.5, 26.9 | 1 |
| | Kigoma, Tanzania (not reported) | Mnyika (2000) [278] | 66 | 705 | 9.4 | 7.2, 11.5 | Giemsa stain microscopy | 6.7 | 4.9, 8.6 | 1 |
| | Blantyre, Malawi (1997-98) | Rogerson (2000) [181] | 2,034 | 4,764 | 42.7 | 41.3, 44.1 | PCR | 41.1 | 39.7, 42.5 | 1 |
| | Kwale, Kenya (1997-98) | (Tobian 2000) [279] | 54 | 102 | 52.9 | 43.3, 62.6 | PCR** | 53.6 | 43.9, 63.3 | 1 |
| | Blantyre, Malawi (1997-99) | Rogerson (2000) [182] | 85 | 339 | 25.1 | 20.5, 29.7 | Giemsa stain microscopy | 30.9 | 26.0, 35.8 | 1 |
| | Medani, Sudan (1997) | Ahmed (2002) [280] | 324 | 550 | 58.9 | 54.8, 63.0 | Giemsa stain microscopy** | 82.9 | 80.0, 86.1 | 1 |
| | Kampala, Uganda (1998) | Kasumba (2000) [281] | 46 | 537 | 8.6 | 6.2, 10.9 | Giemsa stain microscopy** | 5.5 | 3.6, 7.4 | 1 |
| | Manhiça, Mozambique (1998) | Saute (2002) [282] | 156 | 672 | 23.2 | 20.0, 26.4 | Giemsa stain microscopy | 28.0 | 24.6, 31.4 | 10 |
| 2000-2011 | Blantyre, Malawi (2000) | Mankhambo (2002) [283] | 70 | 135 | 51.9 | 43.4, 60.3 | PCR** | 52.3 | 43.8, 60.7 | 1 |
| | Multi- (stable trans), Ethiopia (2000-01) | Newman (2003) [284] | 26 | 249 | 10.4 | 6.6, 14.2 | Giemsa stain microscopy | 0.5* | -0.4, 1.5 | 1 |
| | Multi-(unstable trans), Ethiopia (2000-01) | Newman (2003) [284] | 13 | 713 | 1.8 | 0.8, 2.8 | Giemsa stain microscopy | 8.4 | 4.9, 11.8 | 3 |
| | New Halfa, Sudan (2001-02) | Elghazali (2003) [285] | 15 | 86 | 17.4 | 9.4, 25.5 | Giemsa stain microscopy | 19.1 | 10.8, 27.5 | 1 |
| | Morogoro, Tanzania (2001-02) | Wort (2006) [286] | 494 | 1,684 | 29.3 | 27.1, 31.5 | Giemsa stain microscopy** | 37.4 | 35.1, 39.8 | 1 |
| | Multicentre, Rwanda (2002) | Van Geertruyden (2005) [287] | 195 | 1,432 | 13.6 | 11.8, 15.4 | Giemsa stain microscopy | 13.3 | 11.5, 15.0 | 6 |
| | Blantyre, Malawi (2002-03) | Kalilani (2006) [288] | 372 | 1,172 | 31.7 | 29.1, 34.4 | Giemsa stain microscopy | 41.1 | 38.3, 44.0 | 2 |
| | Multicentre, Kenya (2003) | Parise (2003) [289] | 157 | 339 | 46.3 | 41.0, 51.6 | Giemsa stain microscopy | 63.6 | 58.4, 68.7 | 9 |
| | New Halfa, Sudan (2003-04) | Adam (2005) [290] | 102 | 744 | 13.7 | 11.2, 16.2 | Giemsa stain microscopy | 54.1 | 45.9, 62.3 | 1 |
| | New Halfa, Sudan (2003-04) | Adam (2005) [291] | 57 | 142 | 40.1 | 32.0, 48.2 | Giemsa stain microscopy | 13.4 | 11.0, 15.9 | 1 |
| | Sofala, Mozambique (2003-04) | Montoya (2006) [241] | 78 | 587 | 13.3 | 10.5, 16.0 | Giemsa stain microscopy | 12.8 | 10.1, 15.5 | 6 |
| | Multicentre, Mozambique (2003-04) | Brentlinger (2007) [292] | 255 | 684 | 37.3 | 33.7, 40.9 | Giemsa stain microscopy | 49.7 | 45.9, 53.4 | 5 |
| | Kisumu, Kenya (2003-04) | Ouma (2007) [293] | 123 | 684 | 18.0 | 15.1, 20.9 | Giemsa stain microscopy | 20.0 | 17.0, 23.0 | 1 |
| | Entebbe, Uganda (2003-05) | Hillier (2008) [294] | 268 | 2,459 | 10.9 | 9.7-, 2.1 | Giemsa stain microscopy | 9.1 | 7.9, 10.2 | 1 |
| | Masindi, Uganda (2003-04) | Ndyomugyenyi (2008) [295] | 281 | 802 | 35.0 | 31.7, 38.4 | Giemsa stain microscopy | 46.2 | 42.8, 49.7 | 1 |
| | Mangochi, Malawi (2003-06) | Rantala (2010) [296] | 51 | 475 | 10.7 | 7.9, 13.5 | PCR | 2.1 | 0.8, 3.4 | 1 |
| | Manhiça, Mozambique (2003-05) | Serra-Casas (2011) [297] | 120 | 399 | 30.0 | 25.5, 34.5 | PCR | 25.7 | 21.4, 30.0 | 1 |
| | Southern Malawi (2004-05) | Van Den Broek (2009) [108] | 572 | 2,297 | 24.9 | 23.1, 26.7 | Giemsa stain microscopy | 30.6 | 28.7, 32.5 | 4 |
| | Mbarara, Uganda (2006-09) | Piola (2009) [298] | 329 | 1197 | 27.5 | 25.0, 30.0 | Giemsa stain microscopy | 34.6 | 31.9, 37.3 | 1 |
| | Mukono, Uganda (not reported) | Mbonye (2008) [299] | 573 | 2,344 | 24.5 | 22.7, 26.2 | Giemsa stain microscopy | 30.0 | 28.1, 31.8 | 3*** |
| | Multicentre, Mozambique (2004) | Lujan (2008) [244] | 53 | 1,117 | 4.7 | 3.5, 6.0 | Giemsa stain microscopy | 0.5* | 0.1, 1.0 | 2 |

PCR = Polymerase chain reaction
* The adjusted point prevalence estimate was equal to zero with no variance and is not shown in corresponding figure
** Measured at delivery
*** ANC delivered through facilities and out-reach teams

## Table 4.7 Peripheral malaria point estimates: sub-Saharan Africa (continued)

| Site (year study conducted) | | Reference (year of publication) | No. women positive | No. women tested | Uncorrected prevalence (%) | 95% CI | Diagnostic method | Corrected prevalence (%) | 95% CI | No. ANC facilities |
|---|---|---|---|---|---|---|---|---|---|---|
| **West and Central Africa** | | | | | | | | | | |
| 1990-1999 | North Kivu, DRC (NR) | Meuris (1993) [300] | 80 | 461 | 17.4 | 13.9, 20.8 | Giemsa stain microscopy | 19.0 | 15.4, 22.6 | 1 |
| | Freetown, Sierra Leone (NR) | Morgan (1994) [301] | 142 | 768 | 18.5 | 15.7, 21.2 | Giemsa stain microscopy | 20.8 | 17.9, 23.6 | 1 |
| | Ibadan, Nigeria (NR) | Achidi (1997) [302] | 26 | 116 | 22.4 | 14.8, 30.0 | Giemsa stain microscopy** | 26.8 | 18.7, 34.9 | 1 |
| | Dabou, Côte d'Ivoire (NR) | Watson (1998) [303] | 11 | 26 | 42.3 | 23.3, 61.3 | Giemsa stain microscopy | 57.4 | 38.4, 76.4 | 1 |
| | Multicentre, Nigeria (1992) | Egwunyenga (1997) [304] | 292 | 656 | 44.5 | 40.7, 48.3 | Giemsa stain microscopy** | 60.8 | 57.1, 64.5 | 3 |
| | Farafenni, Gambia (1994) | Menendez (1994) [305] | 72 | 262 | 27.5 | 22.1, 32.9 | Giemsa stain microscopy | 34.6 | 28.8, 40.4 | NA*** |
| | Bauchi, Nigeria (1994-95) | Egwunyenga (1996) [306] | 181 | 830 | 21.8 | 19.0, 24.6 | Giemsa stain microscopy | 25.9 | 22.9, 28.9 | 1 |
| | Jos, Nigeria (1994-95) | Egwunyenga (1996) [306] | 150 | 652 | 23.0 | 19.8, 26.2 | Giemsa stain microscopy** | 27.7 | 24.3, 31.1 | 1 |
| | Eku, Nigeria (1994-95) | Egwunyenga (1996) [306] | 74 | 423 | 17.5 | 13.9, 21.1 | Giemsa stain microscopy** | 19.2 | 15.5, 23.0 | 1 |
| | Kassena-Nankana, Ghana (1994-95) | Browne (2001) [307] | 527 | 928 | 56.8 | 53.6, 60.0 | PCR | 58.3 | 55.1, 61.5 | 17 |
| | Yaounde, Cameroon (1994) | Tako (2005) [308] | 405 | 1,895 | 21.4 | 19.5, 23.2 | Giemsa stain microscopy | 25.2 | 23.2, 27.1 | 2 |
| | Multicentre, Cameroon (1995-98) | Zhou (2002) [309] | 334 | 719 | 46.5 | 42.8, 50.1 | Giemsa stain microscopy | 63.7 | 60.3, 67.3 | 2 |
| | Yaounde, Cameroon (1995-98) | Walker-Abbey (2005) [310] | 77 | 278 | 27.7 | 22.4, 33.0 | Giemsa stain microscopy | 34.9 | 29.3, 40.5 | 3 |
| | Yaounde, Cameroon (1996-2001) | Tako, (2005) [308] | 297 | 1,866 | 15.9 | 14.3, 17.6 | Giemsa stain microscopy | 16.8 | 15.1, 18.5 | 2 |
| | Jos, Nigeria (1997-98) | Egwunyenga (2001) [311] | 816 | 2,104 | 38.8 | 36.7, 40.9 | Giemsa stain microscopy | 52.0 | 49.8, 54.1 | 3 |
| | Ebolowa, Cameroon (1997-98) | Salihu (2002) [312] | 55 | 125 | 44.0 | 35.3, 52.7 | Giemsa stain microscopy | 60.0 | 51.4, 68.6 | 1 |
| | Bansang, Gambia (1997) | Okoko (2002) [313] | 116 | 313 | 37.1 | 31.7, 42.4 | Giemsa stain microscopy | 49.3 | 43.8, 54.9 | 1 |
| | Lagos, Nigeria (1998) | Anorlu (2001) [314] | 174 | 477 | 36.5 | 32.2, 40.8 | Giemsa stain microscopy | 48.4 | 43.9, 52.9 | 1 |
| | Agogo, Ghana (1998) | Mockenhaupt (2008) [315] | 335 | 530 | 63.2 | 59.1, 67.3 | PCR** | 66.1 | 62.1, 70.1 | 1 |
| | Sagamu, Nigeria (NR) | Sule-Odu (2002) [316] | 140 | 564 | 24.8 | 21.3, 28.4 | Giemsa stain microscopy** | 30.5 | 26.7, 34.3 | 1 |
| | Lagos, Nigeria (1999) | Okwa (2003) [274] | 480 | 800 | 60.0 | 56.6, 63.4 | Giemsa stain microscopy | 84.6 | 82.1, 87.1 | 4 |
| | Fako, Cameroon (1999-2001) | Achidi (2005) [317] | 511 | 1,143 | 44.7 | 41.8, 47.6 | Giemsa stain microscopy | 61.1 | 58.3, 63.9 | 1 |
| | Fako, Cameroon (1999-2000) | Akum (2005) [318] | 248 | 735 | 33.7 | 30.3, 37.2 | Giemsa stain microscopy** | 44.2 | 40.6, 47.8 | 1 |

NR = Not reported
** Measured at delivery
*** ANC delivered as part of a national village-based system

**Figure 4.7a Peripheral malaria pooled estimates: East and Southern Africa**

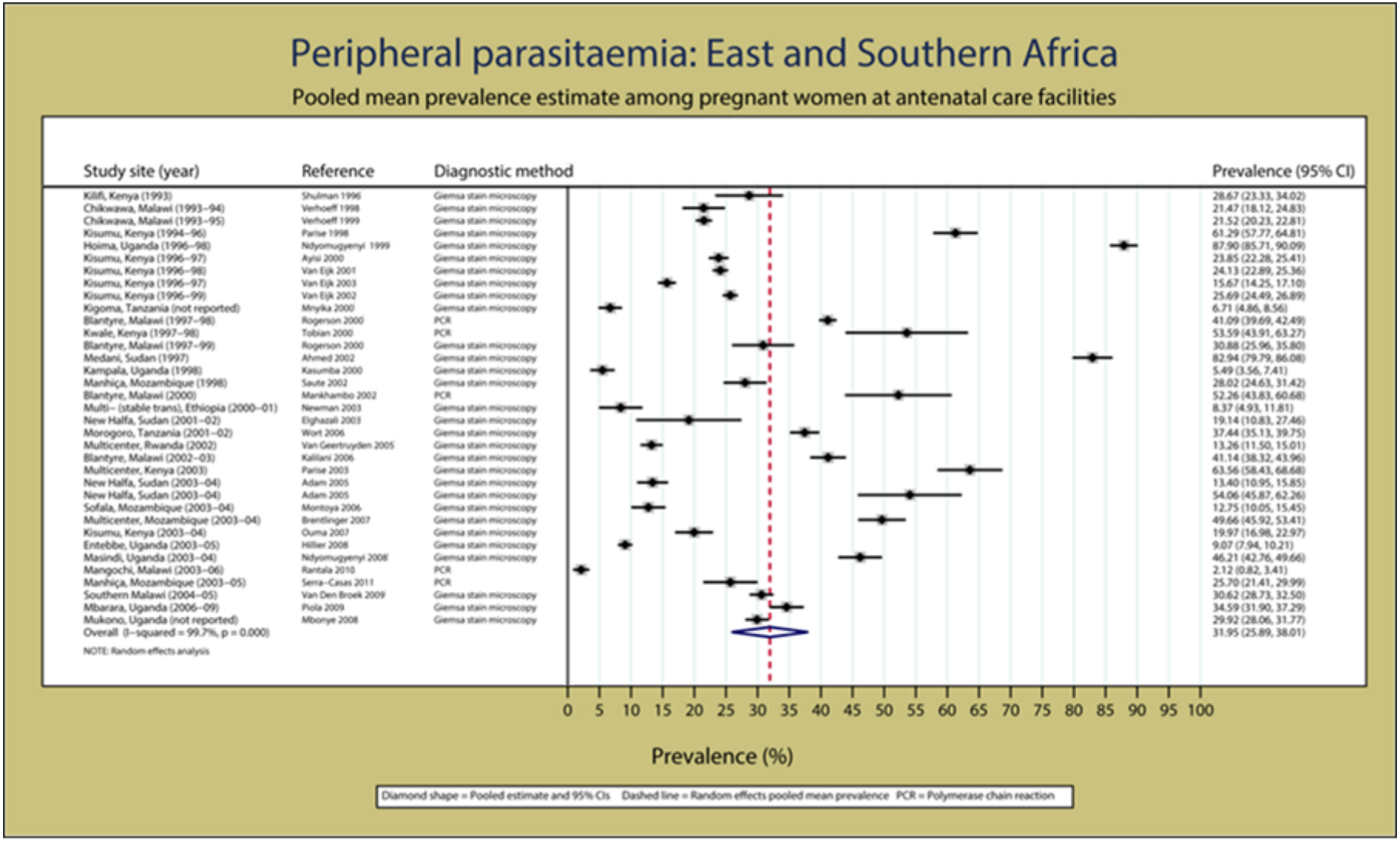
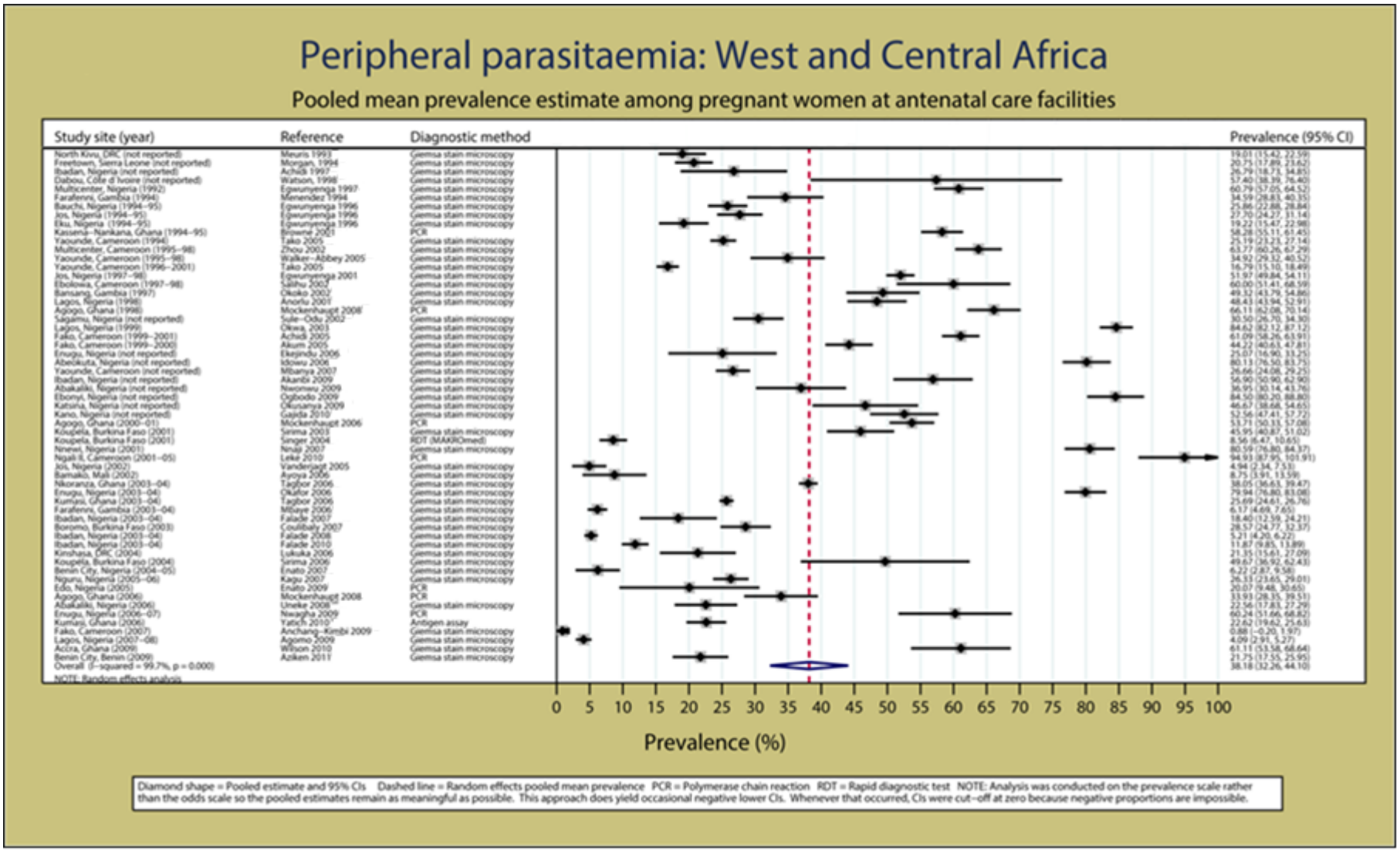


**Figure 4.7b Peripheral malaria pooled estimates: West and Central Africa**



**Placental malaria prevalence**

As for placental malaria, the pooled prevalence in East and Southern Africa was 25.8% (95% CI: 19.7, 31.9) (Figure 4.8a). The highest point estimate, 74.7% (95% CI: 65.5, 84.0), from Kisumu, Kenya was based on histological diagnosis[319] as shown in Table 4.8. In West and Central Africa, the pooled prevalence was 39.9% (95% CI: 34.2, 45.7) (Figure 4.8b) with the highest point prevalence of 91.6% (95% CI: 84.8, 94.4) based on placental histology as well as blood and impression smears from Ebolowa, Cameroon[165] as shown in Table 4.8.

## Table 4.8 Placental malaria point estimates at ANC: sub-Saharan Africa

| Site (year study conducted) | | Reference (year of publication) | No. women positive | No. women tested | Uncorrected prevalence (%) | 95% CI | Diagnostic method | Corrected prevalence (%) | 95% CI | No. ANC facilities |
|---|---|---|---|---|---|---|---|---|---|---|
| **East and Southern Africa** | | | | | | | | | | |
| **1990-1999** | Kisumu, Kenya (1996-99) | Van Eijk (2002) [277] | 475 | 2,502 | 19.0 | 17.4, 20.5 | Placental impression and blood smears with microscopy | 20.0 | 18.4, 21.6 | 1 |
| | Blantyre, Malawi (1997-99) | Rogerson (2000) [182] | 77 | 232 | 33.2 | 27.1, 39.2 | Placental impression and blood smears with microscopy | 35.8 | 29.6, 41.9 | 1 |
| | Kampala, Uganda (1998) | Kasumba (2000) [281] | 36 | 537 | 6.7 | 4.6, 8.8 | Placental blood microscopy | 8.5 | 6.1, 10.8 | 1 |
| **2000-2011** | Blantyre, Malawi (NR) | Rogerson (2003) [320] | 124 | 464 | 26.7 | 22.7, 30.8 | Placental histology, impression, smears with microscopy | 28.6 | 24.5, 32.7 | 1 |
| | Multicentre, Kenya (NR) | Parise (2003) [289] | 163 | 726 | 22.5 | 19.4, 25.5 | Placental blood microscopy | 34.1 | 30.6, 37.5 | 9 |
| | Muheza, Tanzania (2002-05) | Kabyemela (2008) [321] | 55 | 445 | 12.4 | 9.3, 15.5 | Placental impress and blood smears with microscopy | 25.8 | 19.7, 32.0 | 1 |
| | Kisumu, Kenya (2003) | Kassam (2006) [319] | 58 | 85 | 68.2 | 58.3, 78.1 | Placental histology, impression, smears with microscopy | 74.7 | 65.5, 84.0 | 3 |
| | New Halfa, Sudan (2003-07) | Adam (2007) [322] | 94 | 293 | 32.1 | 26.7, 37.4 | Placental histology, impression, smears with microscopy | 34.5 | 29.1, 40.0 | 1 |
| | Manhiça, Mozambique (2003-05) | Serra-Casas (2011) [297] | 110 | 343 | 32.1 | 27.2, 37.0 | PCR | 9.3 | 6.2, 12.4 | 1 |
| | Chikwawa, Malawi (2004-05) | Senga (2007) [323] | 124 | 636 | 19.5 | 16.4, 22.6 | Placental blood microscopy and tissue histology | 20.6 | 17.4, 23.7 | 1 |
| | Gadarif, Sudan (2007-08) | Abdelrahim (2009) [324] | 26 | 150 | 17.3 | 11.3, 23.4 | Placental histology, impression, smears with microscopy | 18.2 | 12.0, 24.3 | 1 |
| | Gadafir, Sudan (2007-08) | Adam (2009) [325] | 46 | 236 | 19.5 | 14.4, 25.5 | Placental histology, impression, smears with microscopy | 20.6 | 15.4, 25.7 | 1 |
| **West and Central Africa** | | | | | | | | | | |
| **1990-1999** | North Kivu, DRC | Meuris (1993) [300] | 242 | 461 | 52.5 | 47.9, 57.1 | Placental histology | 57.0 | 52.5, 61.5 | 1 |
| | Ebolowa, Cameroon (1991-92) | Cot (1995) [165] | 37 | 64 | 57.8 | 45.7, 69.9 | Placental blood microscopy | 91.6 | 84.8, 98.4 | 1 |
| | Farafenni, Gambia (1994) | Menendez (1994) [305] | 116 | 198 | 58.6 | 51.7, 65.4 | Placental blood microscopy and tissue histology | 64.0 | 57.3, 70.7 | NA*** |
| | Basse, Gambia (NR) | Rasheed (1995) [326] | 60 | 97 | 61.9 | 52.2, 71.5 | Placental blood microscopy and tissue histology | 67.6 | 58.3, 76.9 | 1 |
| | Yaounde, Cameroon (1995-98) | Walker-Abbey (2005) [310] | 229 | 278 | 82.4 | 77.9, 86.9 | Placental blood microscopy | Adjusted prevalence >100%** | | 3 |
| | Yaounde, Cameroon (1996-2001) | Tako (2005) [308] | 371 | 1,866 | 19.9 | 18.1, 21.7 | Placental impression and blood smears with microscopy | 21.0 | 19.1, 22.8 | 2 |
| | Bansang, Gambia (1997) | Okoko (2002) [313] | 160 | 313 | 51.1 | 45.6, 56.7 | Placental blood microscopy and tissue histology | 55.7 | 50.2, 61.2 | 1 |
| | Guediawaye, Senegal (1998-99) | N'dao (2003) [327] | 674 | 8,310 | 8.1 | 7.5, 8.7 | Placental blood microscopy | 10.8 | 10.1, 11.4 | 1 |
| | Dakar, Senegal (1998-99) | N'dao (2006) [327] | 785 | 8,273 | 9.5 | 8.9, 10.1 | Placental impression and blood smears with microscopy | 9.4 | 8.8, 10.1 | 1 |
| **2000-2011** | Fako, Cameroon (1999-2001) | Akum (2005) [318] | 233 | 711 | 32.8 | 29.3, 36.2 | Placental impression and blood smears with microscopy | 23.0 | 20.6, 25.4 | 1 |
| | Fako, Cameroon (1999-2001) | Achidi (2005) [317] | 248 | 1,143 | 33.7 | 19.3, 24.1 | Placental impression and blood smears with microscopy | 35.3 | 31.8, 38.8 | 1 |
| | Agogo, Ghana (2000-01) | Mockenhaupt (2006) [328] | 495 | 839 | 59.0 | 55.7, 62.3 | PCR | 47.5 | 44.1, 50.9 | 1 |
| | Koupéla, Burkina Faso (2001) | Sirima (2003) [72] | 19 | 61 | 31.1 | 19.5, 42.8 | Placental blood microscopy | 48.2 | 35.7, 60.8 | 6 |
| | Koupela, Burkina Faso (2001) | Singer (2004) [329] | 247 | 484 | 51.0 | 46.6, 55.5 | PCR | 36.2 | 31.9, 40.5 | 2 |
| | Ngali II, Cameroon (2001-05) | Leke (2010) [330] | 25 | 36 | 69.4 | 54.4, 84.5 | Placental histology, impression, smears with microscopy | 76.1 | 62.1, 90.0 | 1 |
| | Kumasi, Ghana (2003) | Owens (2006) [331] | 33 | 104 | 31.7 | 22.8, 40.6 | Placental blood microscopy and tissue histology | 34.2 | 25.0, 43.3 | 1 |
| | Multicentre, Nigeria (2003-04) | Mokuolu (2009) [332] | 267 | 1,875 | 14.2 | 12.7, 15.8 | Placental blood microscopy and tissue histology | 14.7 | 13.1, 16.3 | 4 |
| | Ibadan, Nigeria (2003-04) | Falade (2007) [333] | 29 | 171 | 17.0 | 11.3, 22.6 | Method not reported | - | - | 1 |
| | Ibadan, Nigeria (2003-04) | Falade (2010) [334] | 128 | 983 | 13.1 | 11.0, 15.2 | Placental blood microscopy | 18.7 | 16.3, 21.2 | 1 |
| | Koupéla, Burkina Faso (2004) | Sirima (2006) [180] | 18 | 59 | 30.5 | 18.8, 42.3 | Placental blood microscopy | 47.2 | 34.4, 59.9 | 1 |
| | Kinshasa, Dem Rep Congo (2004) | Lukuka (2006) [335] | 42 | 196 | 21.4 | 15.7, 27.2 | Placental impression and blood smears with microscopy | 22.7 | 16.8, 28.6 | 4 |
| | Owerri, Nigeria (2004-05) | Ukaga (2007) [336] | 175 | 586 | 29.9 | 26.2, 33.6 | Placental blood microscopy | 46.1 | 42.1, 50.2 | 2 |
| | Benin City, Nigeria (2004-05) | Enato (2007) [337] | 14 | 199 | 7.0 | 3.5, 10.6 | Placental blood microscopy | 9.0 | 5.0, 13.0 | 1 |
| | Fako, Cameroon (2007) | Anchang-Kimbi (2009) [338] | 185 | 306 | 60.5 | 55.0, 65.9 | Placental histology, impression, smears with microscopy | 66.1 | 60.8, 71.4 | 1 |
| | Benin City, Benin (2009) | Aziken (2011) Aziken | 84 | 371 | 22.6 | 18.3, 26.9 | Placental blood microscopy | 34.4 | 34.2, 45.7 | 1 |

PCR = Polymerase chain reaction
NR = Not reported
** The adjusted point prevalence estimate was greater than 100 with no variance and is not shown in the corresponding figure
*** ANC delivered as part of a national village-based system

**Figure 4.8a Placental malaria at ANC: East and Southern Africa**

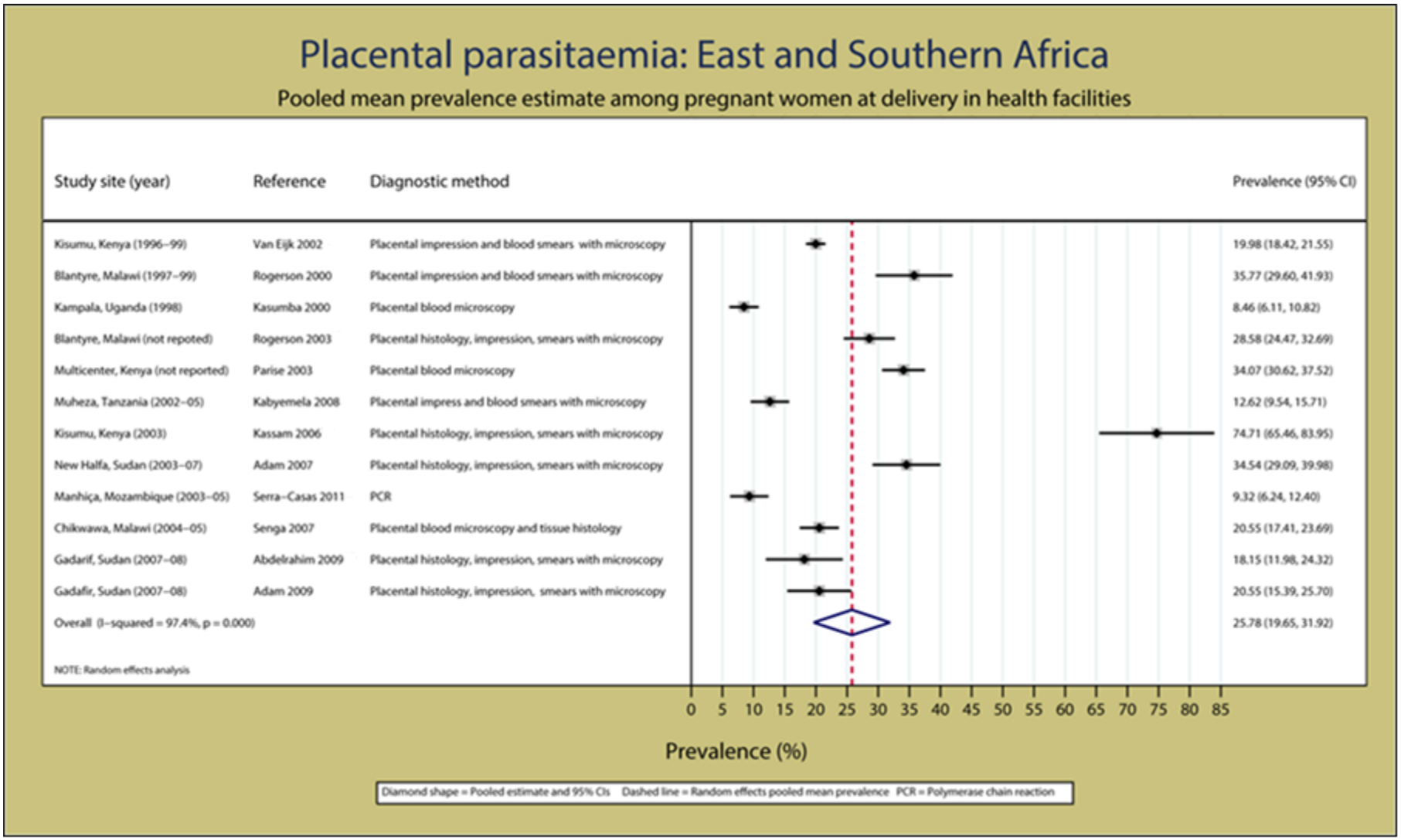


**Figure 4.8b Placental malaria at ANC: West and Central Africa**

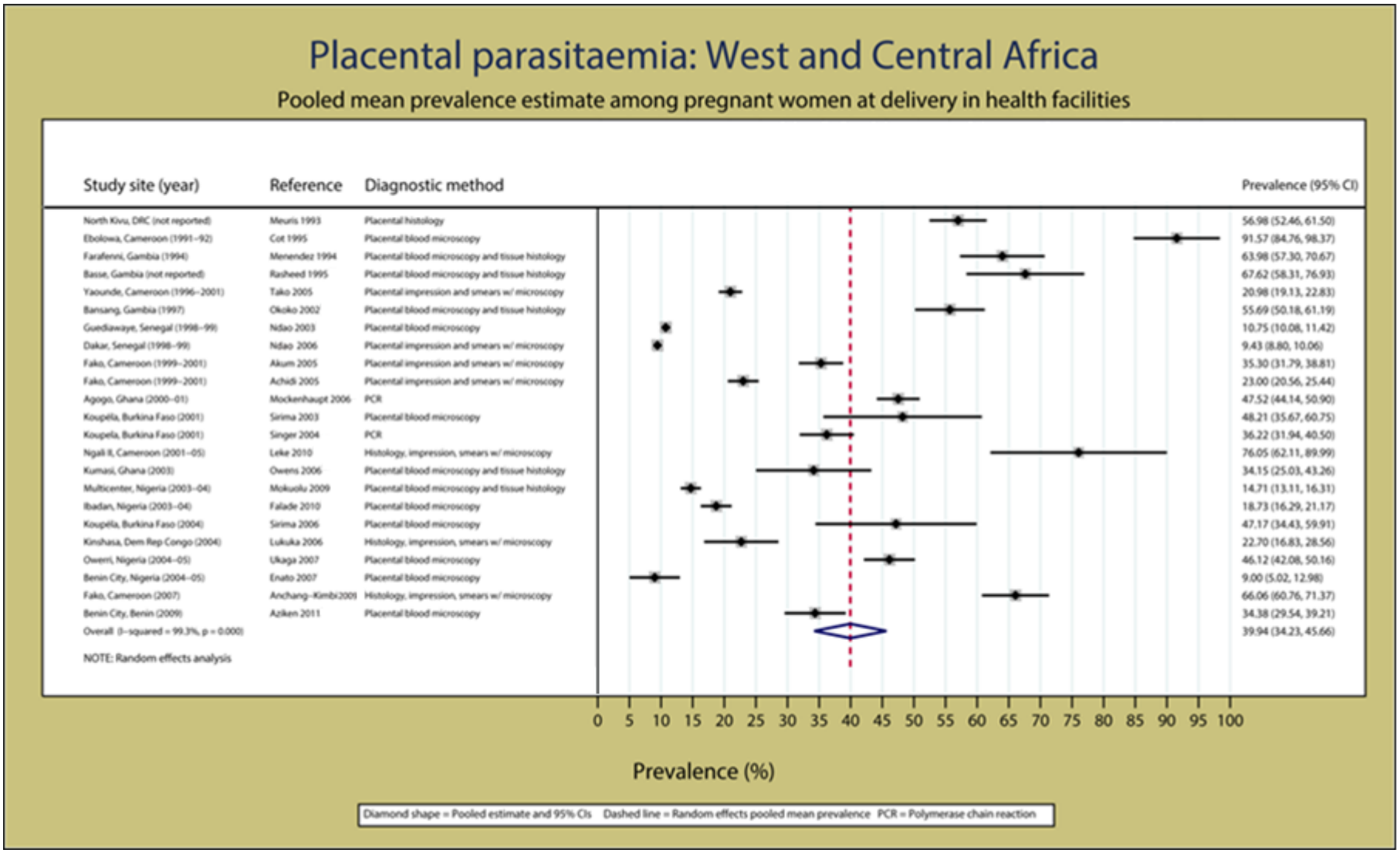


**Table 4.9 Diagnostic methods used: sensitivity and specificity**

| Infection | Diagnostic method | Sensitivity (%) | Specificity (%) | Source |
|---|---|---|---|---|
| **Syphilis** | *Treponema pallidum* haemagglutination (TPHA) only* | ~100 | ~100 | [210] |
| | Rapid plasma reagin (RPR) only | 86-100 | 93-98 | [339] |
| | Venereal disease research laboratory (VDRL) only | 78-100 | 98 | [339] |
| | RPR or VDRL and TPHA | ~100 | ~100 | - |
| | RPR and TPHA | ~100 | ~100 | - |
| | RPR and Determine Syphilis TP | ~100 | ~100 | - |
| | RPR and Microhemagglutination assay–*Treponema pallidum* (MHA-TP) | ~100 | ~100 | - |
| | RPR and Immunoglobulin G (ELISA test) | ~100 | ~100 | - |
| ***Neisseria gonorrhoeae*** | Gram stain | 50-70 | 50-70 | [340] |
| | Culture or gram stain (based on less precise, i.e. mean of gram stain) | 50-70 | 50-70 | - |
| | Polymerase chain reaction (PCR) with urine | 36-75 | 98-100 | [341] |
| | Culture | ~80 | 100 | [342] |
| | Culture and gram stain (based on more precise, i.e. mean of culture) | ~80 | 100 | - |
| | Ligase chain reaction (LCR) with cervical swab | 95-100 | 98-100 | [342] |
| | PCR with cervical swab | 89-97 | 94-100 | [342] |
| ***Chlamydia trachomatis*** | Indirect immunofluorescence | 46-64 | 94-98 | [343] |
| | Culture cervical swab | 74-90 | 98-99 | [342] |
| | Enzyme immunoassay (EIA) | 71-87 | 97-99 | [342] |
| | Culture cervical swab or EIA (based on the less precise, i.e. mean of EIA) | 71-87 | 97-99 | - |
| | PCR urine | 78-89 | 99-100 | [341] |
| | LCR cervical swab | 90-97 | 99-100 | [342] |
| | PCR cervical swab | 99 | 99-100 | [342] |
| | DNA ID-assay cervical swab | ~100 | ~100 | - |
| ***Trichomonas vaginalis*** | Wet-mount microscopy | 38-82 | 100 | [342] |
| | In-pouch culture | 96 | 100 | [344] |
| **Bacterial vaginosis** | Clue cells $\geq$ 20% of epithelium | 40 | 97 | [345] |
| | Unspecified clue cell count | NA | NA | - |
| | Amsel criteria: 3 or 4 of 4 | 51 | 98 | [345] |
| | Spiegel criteria | 75 | 100 | [346] |
| | Nugent score $\geq$7 of 10 | 86-89 | 94-96 | [347,348] |

* TPHA is a confirmatory assay and is not recommended for use alone. Authors of the one paper who reported using TPHA alone wrote that a positive TPHA was considered to be related only to syphilis.[210] Thus, sensitivity and specificity of TPHA only, in this epidemiological context, are assumed to be similar to RPR and TPHA or VDRL and TPHA.

**Table 4.9 Diagnostic methods used: sensitivity and specificity (continued)**

| Infection | Diagnostic method | Sensitivity (%) | Specificity (%) | Source |
|---|---|---|---|---|
| **Peripheral parasitaemia** | Giemsa stain microscopy | 50-90 | ~95 | [349-351] |
| | Polymerase chain reaction | 91 | 91 | [296] |
| | Antigen assay | (reported as equal to microscopy) | | |
| | Rapid diagnostic test (as reported) | 89 | 76 | - |
| **Placental parasitaemia** | Diagnostic method not reported | NA | NA | - |
| | Placental blood microscopy | 63 | 98-99 | [320] |
| | Polymerase chain reaction | 93-99 | 73-76 | [352] |
| | Placental histology | 91 | 98-99 | [320] |
| | Placental blood microscopy and tissue histology (based on histology) | 91 | ~100 | - |
| | Placental histology, impression, smears w/ microscopy (based on histology) | 91 | ~100 | - |
| | Placental impression and blood smears with microscopy (based on impression) | 91 | ~100 | [338] |

## Discussion

This systematic review and meta-analysis of malaria infection and curable STIs/RTIs spanning a 20-year period suggests the prevalence remains high at the population level amongst pregnant women attending ANC facilities in sub-Saharan Africa. The GRADE method of assessing data quality that is commonly applied to evidence from clinical trials was not considered appropriate for this analysis. GRADE requires ranking evidence by outcomes of interest, scored as high, intermediate, low, or very low. Such, 'quality scores' could have been assigned to point prevalence estimates, but that could have introduced a degree of subjectivity. In this context, quality ranking in the context of prevalence estimates was driven by the diagnostic method used rather than any other factor. Thus, an objective alternative was used: the precision of each point prevalence estimate was adjusted using a standard method for correcting errors of known magnitude before incorporating data into the random effects models. This method has been used previously with prevalence data from Tanzania to compare STI/RTI estimates derived by disparate diagnostic tests. Uncorrected and corrected point prevalence estimates in this meta-analysis are shown by study in prevalence Tables 4.2 to 4.8.

Diagnostic methods have changed for many pathogens over the period covered in the systematic review. In recent years, for example, the advent of molecular tests such as PCR and LCR (ligase chain reaction) has allowed researchers to detect infection where older diagnostic methods may have failed to do so. Although statistical adjustments for diagnostic errors can make data more comparable, the pooled prevalence of curable STIs/RTIs and malaria infection is still probably an underestimate of the true

burden of disease. The degree to which this occurred is a function the specific infection and the assay used in diagnosis.

Amongst syphilis studies, 45 of 59 used the non-treponemal RPR or VDRL (venereal disease research laboratory) tests confirmed by a *Treponema*-specific test, reflecting wide acceptance of the diagnostic gold standard. However, because biological false-positive non-treponemal tests can be produced by concurrent malaria infection, as well as other infections such as bejel *(T. pallidum* subspecies *endemicum)*, pinta *(T. pallidum* subspecies *carateum)*, and yaws *(T. pallidum* subspecies *pertenue)*, the burden of syphilis in 15 studies – one-quarter of all data syphilis points – that did not use treponemal confirmatory testing may be overstated. Corrected prevalence estimates for these studies are lower are than the uncorrected numbers originally reported.

The most common assay used to test for *N. gonorrhoeae* was culture which tends to under-estimate true prevalence. This was corrected in 14 studies, slightly increasing those prevalence estimates. The next most frequent assay used was gram stain. The eight studies that employed this method overstated prevalence; gram stain methods have poor sensitivity and specificity in low prevalence settings and among women. Thus, gram stain will routinely overstate the true prevalence in an ANC setting; the method is more appropriate to use at STI clinics with ureteral samples from men. Prevalence estimates based on DNA amplification methods (PCR and LCR) were often corrected downward, which may be the result of detecting non-viable fragments of bacteria (personal communication; Professor David Mabey). An exception is PCR diagnosis of *N. gonorrhoeae* with urine samples from women; the assay has been found

to under-estimate true prevalence (personal communication; Professor Rosanna Peeling) that resulted in an upward correction.

Compared to assays used to for other infections in the systematic review, the tools to detect *C. trachomatis* were most likely captured the true prevalence. Of 24 total studies, enzyme immune-assays were used in 14 studies and eight studies employed DNA amplification. When corrected, prevalence estimates were commonly lower.

Wet-mount microscopy, as previously noted, was used in all but two studies reporting trichomoniasis prevalence, a method that notoriously lacks sensitivity compared to culture or molecular tests.[353,354] Corrected prevalence estimates were nearly twice as high as uncorrected measures. In contrast, in-pouch methods are very precise, resulting in only minor adjustments to two corrected estimates.

Bacterial vaginosis was diagnosed using four different methods: (i) Nugent's score, (ii) Spiegel's criteria, (iii) Amsel's criteria, and (iv) clue cell prevalence estimates. Nugent's score is the preferred method and was applied in studies reporting the seven highest rates of infection in East and Southern Africa. Correction of Nugent's score prevalence estimates were slightly increased in high prevalence areas, but reduced where the uncorrected prevalence was single digit. The other methods – Spiegel's criteria (n = 1), Amsel's criteria (n = 8), and clue cell count (n =5 ) – are not nearly as precise as Nugent's scoring. Consequently, the corrected prevalence for these assays was nearly double the uncorrected measures.

Even with correction, peripheral parasitaemia may be under-represented in this meta-analysis for two reasons. Prior reports of malaria in pregnancy have described the

peak prevalence as occurring between weeks 9-16, declining until term. Indeed, secondary analyses of data from Zambia as part of the third research question found the highest prevalence of malaria infection to be amongst pregnant women who enrolled in the study during gestational week 15. Despite this known variation over the course of pregnancy, studies identified through systematic review did not report the specific gestational age of participants when peripheral parasitaemia was measured. Another important source of under-estimated disease burden comes from the fact that most malaria diagnoses were based on microscopy without molecular-test correction. A study of pregnant women in Blantyre, Malawi, illustrates the extent to which under-diagnosis may occur with conventional microscopy; peripheral parasitaemia was found in 51.9% (43.4, 60.3) of women using PCR, while microscopy only detected infection in 14.9% (11.8, 18.0) of pregnant women.[283] Overall, peripheral malaria was measured by microscopy only in 63 studies whereas 10 studies included PCR. Corrected microscopy estimates were higher than uncorrected measures. In contrast, PCR methods were corrected downward slightly, potentially accounting for an overstated prevalence attributable to the detection of non-viable parasite DNA fragments.

Histology was used to measure placental malaria in over 14 studies, or one-third overall, while microscopy and placental smears were the primary means of measurement in 11 and 8 studies, respectively. In all instances, corrected estimates were higher than uncorrected measures.

**Dual prevalence in the sub-regions**

Overall, pregnant women in East and Southern Africa had a higher prevalence of STIs/RTIs compared to expecting mothers in West and Central Africa. Several factors may contribute to this difference that are related to exposure including: (i) higher prevalence of STIs/RTIs circulating at the community level, (ii) patterns of male circumcision, (iii) sexual behaviour related to age of sexual debut, (iv) age at first marriage, (v) premarital sex, (vi) total lifetime partners, and (vii) concurrent sex partners. The prevalence of HIV, for example, remains substantially higher in East and Southern Africa compared to the West and Central sub-region. Indeed, the Joint United Nations Programme on HIV/AIDS estimates that 34% of the world's HIV-positive population were living in 10 countries of Southern Africa in 2010.[355] Although the AIDS epidemic in East and Southern Africa has declined over the past decade, the prevalence remains higher than in West and Central Africa.

Evidence from this meta-analysis also suggests that peripheral and placental malaria is slightly higher in West and Central Africa compared to the East and Southern sub-region. This may be due to greater seasonal intensity of transmission within West and Central Africa. Recent declines in malaria transmission within selected locales of Africa will likely translate into fewer pregnant women acquiring immunity from one pregnancy to the next. Thus, all women in their reproductive years, rather than just primi- and secundigravidae, may remain vulnerable to the consequences of malaria infection in the years ahead until the absolute risk of exposure is negligible. This may be of particular concern if interest wanes in the provision of IPTp-SP in geographic areas where malaria transmission intensity is very low.

HIV and malaria infection interact to increase placental parasitaemia and the incidence of LBW.[230] Infection by *P. falciparum* also increases viral load amongst HIV-positive pregnancy women.[356] Recent evidence suggests that malaria parasites may be more likely to develop wild-type mutations following exposure to IPTp-SP when administered to HIV-positive women.[357] Despite the known interaction between STIs/RTIs and HIV, presumptive treatment of STIs/RTIs amongst pregnant women in Rakai, Uganda, did not reduce the incidence of maternal to child transmission of HIV.[358] However, malaria chemoprophylaxis was not provided in that study and, therefore, the potential modulating effect of malaria on vertical transmission in the presence of STIs/RTIs remains unknown.

Interestingly, just five publications of the 171 studies of the systematic review reported the prevalence of syphilis and malaria among participants,[108,226,237,241,244] and only one paper reported the incidence of malaria and STI/RTI co-infection, a prospective cohort study of pregnant women in Tanzania. Co-infection was found amongst 48.3% of RPR-positive women who had placental parasitaemia; 35.0% of RPR-negative women had malaria-infected placentas ($P < 0.001$).[146] Separate analysis of the Zambia data used in the third research question showed that 38.7% (95% CI: 35.7, 41.6) of pregnant women were co-infected with malaria parasites and at least one curable STI/RTI, whereas half that number, 18.9% (95% CI: 16.5, 21.2), were infected with malaria parasites only.[202] This strengthens the evidence base for combination treatment that addresses the dual burden of malaria infection and curable STIs/RTIs and, indeed, evidence from Zambia shows that IPTp-SP is protective against this dual burden.

This systematic review and meta-analysis has limitations, not the least of which is the inherent delay between gathering evidence at the field level and publication. This is consequential because HIV-prevention campaigns, including STI/RTI screening and treatment programmes, have made inroads in reducing transmission in many settings. To capture these reductions, more data from the recent past are needed. Another limitation is that, even after stratifying by geography and age of study, heterogeneity amongst studies persisted. Consequently, random effects models were used, rather than fixed effects, but these produced wide confidence intervals in some instances. Finally, evidence was limited to English-language sources and abstracts that had been translated into English.

Considering the 171 studies of this systematic review, there is wide variability in prevalence estimates that has been extracted from a range of populations that cover large geographic areas over a 20-year period. Stratification of data into sub-regions has value for policymakers and programme managers for planning purposes as well as advocacy. Presenting the range of prevalence estimates by infection and sub-region has particular public health value that highlights inequities that merit intervention.

## Q 5. Effect of azithromycin on curable STIs/RTIs in pregnancy

### Methods

The effect of azithromycin monotherapy on malaria infection was summarised in the Literature Review section of this thesis. However, given the high dual burden of malaria infection and curable STIs/RTIs amongst pregnant women, would azithromycin be an efficacious drug against curable STIs/RTIs to be included as part of IPTp? A

systematic review and meta-analysis was conducted to characterise the *in vitro* and *in vivo* effect of azithromycin against the curable STIs/RTIs of interest.

PubMed, MEDLINE and EMBASE were searched between April and May 2013 using medical subject headings and free-text terms as shown in Table 5.1. Searches were limited to the English language. With each query, the infection and causal organism

| Infection | Medical search headings | Free text terms |
|---|---|---|
| **Syphilis** | Topic=("Syphilis" OR "*Treponema pallidum*") | *Treponema pallidum* OR Syphilis AND azithromycin OR macroide |
| ***Neisseria gonorrhoeae*** | Topic=("*Neisseria gonorrhoeae*") OR Topic=(Gonorrhea) OR Topic=(Gonorrhoea) | *Neisseria gonorrhoeae* OR gonorrhoea OR gonorrhea AND azithromycin OR macroide |
| ***Chlamydia trachomatis*** | Topic=("Chlamydia") OR Topic= (trachomatis) | *Chlamydia trachomatis* OR chlamydia AND azithromycin OR macroide |
| ***Trichomonas vaginalis*** | Topic=("*Trichomonas vaginalis*") OR Topic=("Trichomoniasis") | *Trichomonas vaginalis* OR trichomoniasis AND azithromycin OR macroide |
| **Bacterial vaginosis** | Topic=("Bacterial vaginosis") OR Topic=("Vaginalis bacterial") | Bacterial vaginosis OR Vaginalis bacterial AND azithromycin OR macroide |

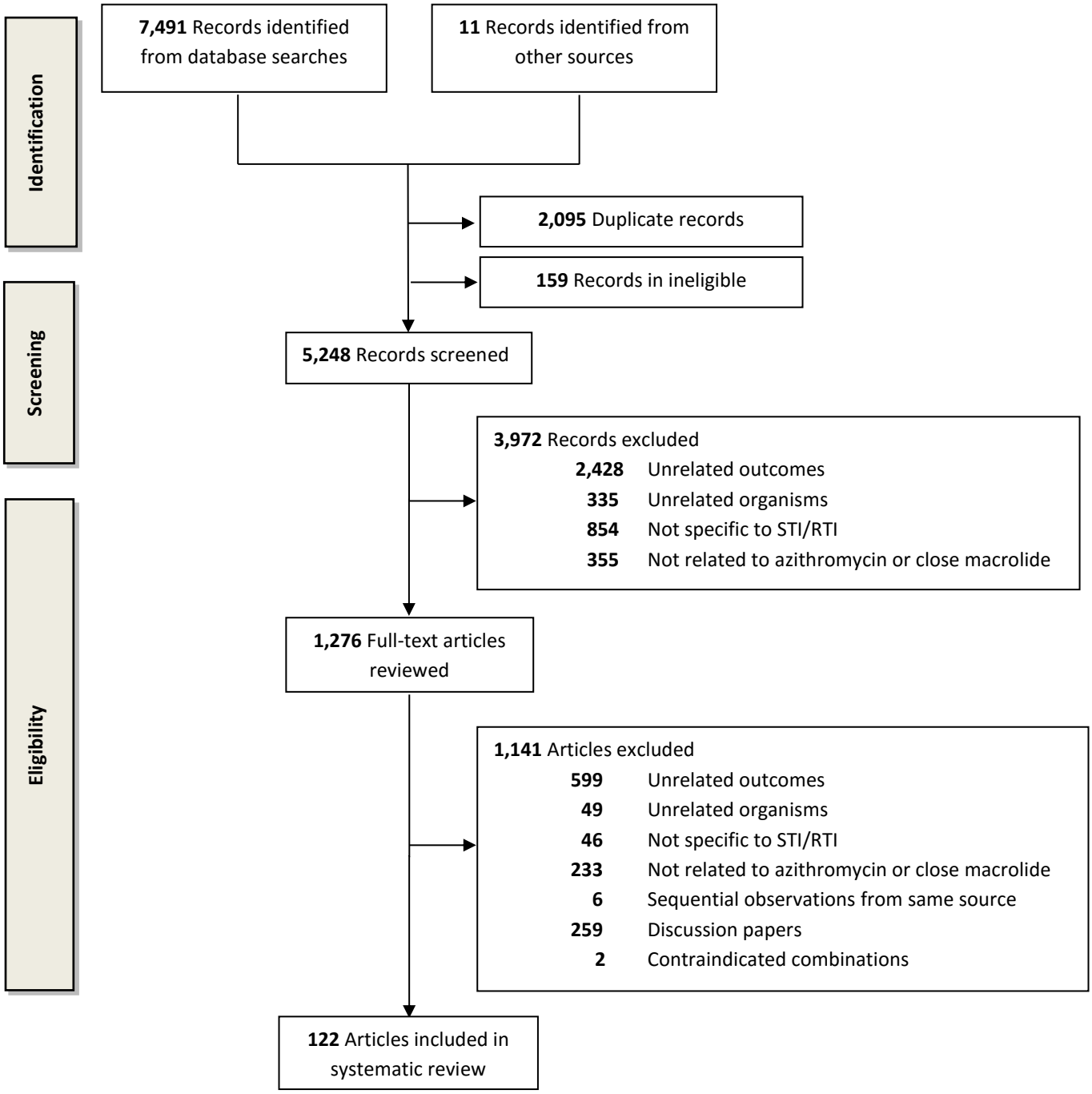
were used together; for example, 'Syphilis' OR '*Treponema pallidum*' were combined with search terms 'azithromycin' OR 'macrolide'. Reference lists were also reviewed for additional documents. As with prior systematic reviews, publication and author names were not blinded, but data abstraction was done in duplicate.

**Table 5.1 Medical subject headings and free text terms**

## Results

As shown in Figure 1 below, a total of 122 articles met inclusion criteria for the systematic review.

**Figure 5.1 PRISMA flowchart for systematic review**



**Syphilis and azithromycin**

***In vivo* evidence**

The WHO recommends treating pregnant women who have a syphilis infection with 2.4 million units (mμ) of benzanthine penicillin G (BPG) administered by intramuscular injection.[109] Thus, the focus of *in vivo* evidence from this systematic review reflects findings from six clinical trials that reported outcomes amongst non-pregnant adults following treatment with BPG, azithromycin, or a combination of BPG and azithromycin as shown in Table 5.2a. The oldest data are from a trial in the United States (1993 to 1997) in which individuals who learned they had been exposed to infectious-stage syphilis through sexual intercourse in the preceding 30 days were given either 1 g azithromycin (n = 40) or BPG (n = 23). Three months post-treatment, RPR and fluorescent treponemal antibody absorption tests were negative for all participants in both treatment groups.[359] Another trial, also in the United States and during the same period of time, was designed to measure treatment outcomes in a population at high risk of contracting STIs/RTIs. Diagnostic methods were not reported, but the trial was suspended after two of the first 12 patients given 1 g azithromycin went on to fail their tests of cure, whereas all 13 participants were cured using BPG ($P = 0.18$).[360] A three-arm trial of early syphilis in the United States then compared treatment outcomes amongst patients given BPG, versus 2 g azithromycin once, versus 2 g azithromycin on two occasions with one week in between doses. RPR and fluorescent treponemal antibody absorption tests showed that cure was achieved in 85.7% (n = 14; 95% CI: 60.0% to 95.7%) of patients given BPG, 94.1% (n = 17; 95% CI: 72.7% to 98.6%) amongst recipients of 2 g azithromycin once and 82.8% (n = 29; 95% CI: 65.3% to 92.3%) in participants who twice received 2 g azithromycin.[361]

## Table 5.2a Syphilis RCTs of azithromycin vs. benzathine penicillin G

*Low-risk and unspecified low-risk populations and high-risk populations*

| Reference | Country | Year(s) | Regimen | No. cured of treated | Percent cured | 95% CI | Diagnostic methods used | Follow up | Stage of infection |
|---|---|---|---|---|---|---|---|---|---|
| **Specified low-risk populations** | | | | | | | | | |
| Hook (2010) [362] | Madagascar<br>United States | 2000-2007 | 2 g azithromycin<br>2.4 mμ BPG | 180/232<br>186/237 | 77.6%<br>78.5% | 71.8% to 82.5%<br>72.8% to 83.2% | RPR and FTA-ABS | 6 months | Primary, secondary, early latent syphilis |
| Kiddugavu (2005) [363] | Uganda | 1994-1998 | 1 g azithromycin<br>2.4 mμ BPG<br>1 g azithromycin plus 2.4 mμ BPG | 55/94<br>66/93<br>221/313 | 58.5%<br>71.0%<br>70.6% | 48.4% to 68.0%<br>61.0% to 79.2%<br>65.3% to 75.4% | TRUST and TPHA (initial TRUST titres ≤ 1:2) | 10 months | Serologic syphilis |
| | | | 1 g azithromycin<br>2.4 mμ BPG<br>1 g azithromycin plus 2.4 mμ BPG | 38/71<br>31/75<br>169/309 | 53.3%<br>41.3%<br>54.7% | 42.0% to 64.7%<br>30.9% to 52.7%<br>49.1% to 60.2% | TRUST and TPHA (initial TRUST titres ≥ 1:4) | 10 months | Serologic syphilis |
| **Unspecified low risk populations** | | | | | | | | | |
| Klausner (2006) [360] | United States | 2004 | 1 g azithromycin<br>2.4 mμ BPG | 10/12<br>13/13 | 83.3%<br>100.0% | 54.6% to 95.0%<br>NA | NR | NR | Exposed to infectious syphilis |
| Hook (2002) [361] | United States | 1995-1997 | 2 g azithromycin<br>2 g azithromycin (x2) week apart 2.4 mμ BPG | 14/14<br>19/22 | 100.0%<br>86.4% | NA<br>66.4% to 95.0% | RPR and MHA-TP or FTA-ABS | 12 months | Primary, secondary, early latent syphilis |
| Hook (1999) [359] | United States | 1995-1997 | 1 g azithromycin<br>2.4 mμ BPG | 40/40<br>23/23 | 100.0%<br>100.0 | NA<br>NA | RPR and FTA-ABS | 3 months | Exposed to infectious syphilis |
| **High-risk population** | | | | | | | | | |
| Riedner (2005) [364] | Tanzania | 2000-2003 | 2 g azithromycin<br>2.4 mμ BPG | 159/163<br>157/165 | 97.5%<br>95.2% | 93.9% to 99.0%<br>90.7% to 97.5% | RPR and PCR | 9 months | Primary, secondary, higher titre latent syphilis |

2.4 mμ BPG = 2.4 million units benzathine penicillin G; RPR = Rapid plasma regain; FTA-AB S= Fluorescent treponemal antibody-absorption; TRUST = Toluidine Red Unheated Serum Test; TPHA = *Treponema pallidum* haemagglutination; MHA-TP = Microhaemagglutination assay-*Treponema pallidum*; PCR = Polymerase chain reaction; NR = Not reported; NA = Not applicable

Three trials have investigated BPG versus azithromycin in sub-Saharan Africa, the first being a community-randomised trial in Uganda (1994 to 1998) amongst non-pregnant adults with serological syphilis. Diagnosis pre- and post-treatment were based on toluidine red unheated serum tests (TRUST) and TPHA assays. Treatment efficacy varied across regimens depending on TRUST titres at enrolment. Amongst patients with initial titres $\leq$ 1:2, BPG cured 71.0% (n = 93; 95% CI: 61.0% to 79.2%) of cases, compared to 58.5% (n = 94; 95% CI: 48.4% to 68.0%) in patients given 1 g azithromycin, and 70.6% (n = 313; 95% CI: 65.3% to 75.4%) of participants treated with 1 g azithromycin plus BPG. If titres at enrolment were $\geq$ 1:4, the efficacy of BPG was reduced to 41.3% (n = 75; 95% CI: 30.9% to 52.7%). Treatment efficacy was also lower amongst groups given azithromycin, but higher than BPG alone. Recipients of 1 g azithromycin alone had a cure rate of 53.3% (n = 71; 95% CI: 42.0% to 64.7%), whereas 1 g azithromycin plus BPG cured 54.7% of cases (n = 309; 95% CI: 49.1% to 60.2%).[363]

These results were followed by a trial in Tanzania (2000 to 2003) amongst patients from high-risk populations. All 328 subjects had a titre of at least 1:8 on RPR tests; 106 had baseline titres of $\geq$ 1:64, levels indicative of active syphilitic lesions. Serological cure was observed in 97.5% (n = 163; 95% CI: 93.9% to 99.0%) of participants treated with 2 g azithromycin versus 95.2% (n = 165; 95% CI: 90.7% to 97.5%) in the BPG group as measured by RPR test and TPPA assays.[364]

The most recent study comparing the efficacy of azithromycin versus BPG is a multi-centre trial (2000 to 2007) in Madagascar (n = 421) and North America (n = 94) amongst HIV-negative participants with early syphilis. Serological cure was reported in 77.6% of subjects given 2 g azithromycin (n = 232; 95% CI: 71.8% to 82.5%) and 78.5%

(n = 237; 95% CI: 72.8% to 83.3%) in the BPG group based on RPR testing. Non-serious adverse events were reported by 61.5% (n = 174; 95% CI: 55.7% to 67.0%) of individuals treated with 2 g azithromycin, most of whom had self-limiting gastrointestinal discomfort. In contrast, 46.1% (95% CI: 40.6% to 52.1%) of BPG recipients reported non-serious adverse events.[362]

***In vitro* evidence**

Fourteen *in vitro* studies met inclusion criteria, seven with isolates from low-risk and unspecified low-risk populations, Table 5.2b, and seven from high-risk or mixed-risk groups, Table 5.2c. The first report to associate azithromycin treatment failure with A→G mutations at the 2058 position of the 23S rRNA gene of *T. pallidum* came from surveillance monitoring in San Francisco.[365] Retrospective analysis revealed that 4.0% (N = 25; 95% CI: 0.9% to 19.6%) of isolates had A→G mutations between 1999 and 2002. In 2003, the proportion of isolates with A→G mutations increased to 36.7% (N = 30; 95% CI: 21.9% to 54.6%); by 2004, 56.1% (N = 66; 95% CI: 44.0% to 67.3%) had selected for resistance.[365] In Dublin, however, 88.2% (N = 17; 95% CI: 65.3% to 96.4%) of isolates already had A→G mutations by 2002.[366]

Macrolide resistance has been strongly associated with prior use in the previous year. Isolates from Seattle (2001 to 2005) were two-times more likely to be resistant if patients had been treated with macrolides in the past 12 months (RR = 2.2; 95% CI: 1.1 to 4.4; $P = 0.02$).[367] This relationship persisted over the decade. Mutations A2058G and A2059G, which are associated with clinical failures of azithromycin, were found in 88.9% (N = 36; 95% CI: 74.6% to 95.5%) of isolates from 2001 to 2010 amongst patients exposed to macrolides in the preceding 12 months, whereas 61.2% (N = 98;

95% CI: 51.3% to 70.4%) of isolates from individuals who had not received prior macrolide treatment contained the same mutations.[368] Similar mutations were found amongst *T. pallidum* isolates from eight cities across China (2008 to 2011). A2058G was present in 97.0% individuals who had taken macrolides in the previous 12 months versus 62.5% of patients who had not (N=211; OR=19.65; 95% CI: 5.8 to 66.9).[369] In Taiwan (2009 to 2011) the opposite was observed; not one single A2058G or A2059G mutation was found amongst 211 isolates tested from a population where only one person had been given macrolide therapy in the previous year.[370] Similarly, there was no evidence of resistance amongst 141 amplified samples from HIV-negative heterosexual patients in Madagascar.[371] Although use of macrolides in the previous year was not reported, the Essential Drugs List of the Malagasy Ministry of Health does not include macrolides.[372]

## Table 5.2b Azithromycin and syphilis: low-risk populations

| Reference | Country | Year(s) | Specimen source | Sample size | Resistant mutation | Number resistant | Percent resistant | 95% CI | Additional details |
|---|---|---|---|---|---|---|---|---|---|
| **Low-risk populations** | | | | | | | | | |
| Chen (2013) [373] | United States | 2007-2009 | Surveillance | 129 | A2058G<br>A2058G<br>A2059G | 83<br>67<br>17 | 64.3%<br>51.9%<br>13.2% | 55.8% to 72.1%<br>43.4% to 60.4%<br>8.4% to 20.1% | From patients with primary of secondary syphilis attending STI clinics |
| Van Damme (2009) [371] | Madagascar | NR | Randomised clinical trial | 186 | 23S rRNA | 0 | 0.0% | NA | DNA of *T. pallidum* was detected in 141 samples; 61% of patients were male; 98% were heterosexual |
| **Unspecified low-risk populations** | | | | | | | | | |
| Müller (2012) [374] | South Africa<br>Lesotho | 2005-2010 | Surveillance | 100 | A2058G<br>A2059G | 1<br>0 | 1.0%<br>0.0% | 0.0% to 5.4% | 117 ulcer specimens collected of which 100 were positive for *T. pallidum*. |
| A2058G Prevalence Workgroup (2012) [375] | United States | 2007-2009 | Surveillance | 141 | A2058G | 75 | 53.1% | 45.0% to 61.2% | From patients with primary of secondary syphilis attending STI clinics; MSM were nearly 6 times more likely to have resistant syphilis compared to heterosexual women and men |
| Matějková (2009) [376] | Czech Republic | 2005-2008 | Passive case detection | 22 | 23S rRNA<br>A2058G<br>A2059G | 8<br>4<br>4 | 36.4%<br>18.2%<br>18.2% | 19.7% to 57.3%<br>7.5% to 38.8%<br>7.5% to 38.8% | |
| Martin (2009) [377] | China | 2007-2008 | Passive case detection | 38 | A2058G | 38 | 100.0% | NA | Patients presenting to STI clinic with symptoms compatible with primary syphilis |
| Lukehart (2004) [366] | United States | | | | | | | | PCR to detect 23S rRNA gene mutations; confirmation of azithromycin resistance was conducted through intradermal rabbit inoculation. |
| | *San Francisco* | 1999-2002 | Surveillance | 25 | 23S rRNA | 1 | 4.0% | 1.0% to 19.6% | |
| | *San Francisco* | 2003 | | 30 | | 11 | 36.7% | 21.9% to 54.6% | |
| | *Seattle* | 2001-03 | | 23 | | 3 | 13.0% | 4.7% to 32.3% | |
| | *Baltimore* | 1998-2000 | | 19 | | 2 | 10.5% | 3.2% to 31.7% | |
| | Ireland | | | | | | | | |
| | *Dublin* | 2002 | Surveillance | 17 | 23S rRNA | 15 | 88.2% | 65.3% to 96.4% | |
| | Multiple locations | 1912-1987 | Historical | 18 | 23S rRNA | 1 | 5.6% | 1.3% to 2.6% | |

rRNA=Ribosomal ribonucleic acid; NA=Not applicable; DNA=Deoxyribonucleic acid; STI=Sexually transmitted infection; MSM=Men who has sex with men; PCR=Polymerase chain reaction

## Table 5.2c Azithromycin and syphilis: high- and mixed-risk populations

| Reference | Country | Year(s) | Specimen source | Sample size | Resistant mutation | Number resistant | Percent resistant | 95% CI | Additional details |
|---|---|---|---|---|---|---|---|---|---|
| **High-risk populations** | | | | | | | | | |
| Tipple (2011) [378] | United Kingdom | 2006-2008 | Cross-sectional | 18 | 23S rRNA<br>A2058G | 12<br>11 | 66.6%<br>61.1% | 43.4% to 83.7%<br>38.4% to 79.7% | Specimens from men, 94.1% were MSM |
| Rekart (2003) [379] | Canada | 2000 | Mass drug administration | 25 | 23S rRNA | 0 | 0.0% | NA | 1.8 g azithromycin given to sex workers and clients (N = 4,384) |
| **Mixed-risk populations** | | | | | | | | | |
| Chen (2012) [369] | China | 2008-2011 | Cross-sectional | 211 | A2058G | 194 | 91.9% | 87.2% to 95.1% | 391 samples collected; 6.1% from FSW, 71.8% reported sex with FSW, and 1.4% were MSM |
| Muldoon (2012) [380] | Ireland | 2009-2010 | Cross-sectional | 29 | A2058G | 27 | 93.1% | 77.9% to 97.9% | 34.6% (36/104) of samples were positive for *T. pallidum* by PCR; 29 sequenced |
| Martin (2010) [381] | Canada | 2007-2009 | Cross-sectional | 43 | A2058G | 7 | 16.3% | 8.2% to 30.1% | 449 samples collected from 374 patients; 43 were positive for *T. pallidum* and sequenced. |
| Mitchell (2006) [382] | United States | 2000-2002 | Retrospective | 25 | 23S rRNA | 1 | 4.0% | 1.0% to 19.6% | Patients (N = 1,308) diagnosed primary or secondary syphilis; all treatment failure and resistance in MSM/bisexual patients |
| | | 2003 | | 32 | | 13 | 40.6% | 25.5% to 57.9% | |
| | | 2004 | | 66 | | 37 | 56.1 | 44.0% to 67.4% | |
| Morshed (2006) [383] | Canada | 2000-2003 | Retrospective | 47 | 23S rRNA | 1 | 2.1% | 0.5% to 11.1% | MSM patients presenting to STI clinic with primary or secondary syphilis |
| | | 2004 | | 9 | | 4 | 44.4% | 18.7% to 73.8% | |

rRNA=Ribosomal ribonucleic acid; MSM=Men who have sex with men; NA=Not applicable; PCR=Polymerase chain reaction; FSW=Female sex worker; STI=sexually transmitted infection

### *Neisseria gonorrhoeae* and azithromycin

#### *In vivo* evidence

The WHO recommends the treatment of pregnant women with *N. gonorrhoeae* infection using 400 mg cefixime as a single dose, or 125 mg ceftriaxone by intramuscular injection.[110] However, azithromycin has been used for the treatment of gonorrhoea amongst non-pregnant adults during the past two decades. Eleven trials were identified by systematic review as shown in Table 5.3a. Nine trials between 1989 and 1999 were conducted amongst attendees of STI clinics using 1 g azithromycin. Three of these trials were open label without comparators,[384-386] whereas six were two-arm trials that compared azithromycin to ciprofloxacin and/or doxycycline.[387-392] Meta-analysis using random effects models[175] suggests that the pooled efficacy of azithromycin against *N. gonorrhoeae* was 97.0% (N = 539; 95% CI: 95.5% to 98.5%). This is slightly higher than 96.5% (N = 539; 95% CI: 94.3% to 97.6%) reported in a 2010 review[393] that added numerators and divided by the sum of denominators amongst the same nine trials. However, the methods used in that review were dubious, failing to account for heterogeneity across study populations and giving equal weight to all trials regardless of their individual study precision. Nevertheless, it is unlikely the same efficacy would be observed today using 1 g azithromycin in high-income countries following 25 years of cumulative drug pressure. However, the epidemiological context in sub-Saharan Africa is different where azithromycin use has been almost exclusively limited to trachoma eradication campaigns.[394]

Two RCTs investigated the use of 2 g azithromycin amongst patients at STI clinics. The first was a multi-centre trial in the United States (1991 and 1992) in which 98.9%

(N=374; 95% CI: 97.3% to 99.6%) of patients were cured.[395] A similar RCT in New Delhi (2005 and 2006) involved 42 participants. The reported loss to follow up was high, 52.4%, but all 22 subjects who returned for a test of cure had their *N. gonorrhoeae* infections cleared.[396]

## Table 5.3a *Neisseria gonorrhoeae* RCTs of azithromycin

| Reference (year of publication) | Country | Year(s) | Azithromycin dose | No. of cured evaluated | Percent cured | 95% CI | Diagnostic methods | Additional details |
|---|---|---|---|---|---|---|---|---|
| **Dose of 2 g azithromycin** | | | | | | | | |
| Khaki (2007) [396] | India | 2005-2006 | 2 g | 22/22 | 100% | NA | Gram stain<br>TOC days 5-7 | |
| Handsfield (1994) [395] | United States | 1991-1992 | 2 g | 370/374 | 98.9% | 97.3% to 99.6% | | |
| **Dose of 1 g azithromycin** | | | | | | | | |
| Rustomjee (2002) [392] | South Africa | 1999 | 1 g | 30/31 | 96.8% | 83.8% to 99.2% | LCR for NG/CT<br>TOC day 14 | 100% (n = 21) NG infections cured<br>100% (n = 14) CT infections cured<br>90.0% (n = 10; 95% CI: 58.7 to 97.8%) co-infections cured |
| Swanston (2001) [386] | Trinidad and Tobago | 1996 | 1 g | 125/127 | 98.4% | 94.5% to 99.5% | ELISA for NG Culture for CT;<br>TOC days 7-10 | 100% (n = 115) NG infections cured<br>95.7% (n = 23; 95% CI: 78.9 to 99.0%) CT infections cured<br>88.3% (n = 12; 95% CI: 54.6 to 95.0%) co-infections cured |
| Gruber (1997) [391] | Croatia | 1994-1995 | 1 g | 48/50 | 96.0% | 86.5% to 98.8% | Culture and gram-stain for NG; TOC day 14 | |
| Gruber (1995) [390] | Croatia | 1991-1993 | 1 g | 24/24 | 96.0% | 80.4% to 99.1% | Culture and gram-stain for NG; TOC day14 | |
| Steingrimsson (1994) [389] | Iceland | NR | 1 g | 27/28 | 96.4% | 82.4% to 99.2% | Culture for NG w/ DFA;<br>Culture for CT; TOC day 28 | 92.4% (n = 79; 84.4 to 96.4%) CT infections cured |
| Waugh (1993) [385] | United Kingdom | 1990-1991 | 1 g | 85/89 | 95.5% | 89.0% to 98.2% | Culture and gram-stain for NG; Culture for CT;<br>TOC day 10 | 100% (n = 22) NG/CT co-infections cured |
| Odugbemi (1993) [384] | Nigeria | 1989-1990 | 1 g | 114/120 | 95.0% | 89.5% to 97.6% | Culture for NG; TOC day 14 | |
| Steingrimsson (1990) [388] | Iceland | NR | 1 g (day 0) | 11/12 | 91.7% | 64.0% to 98.1% | Culture for NG/CT;<br>TOC day 28 | 97.7% (n = 44; 88.2 to 99.5%) CT infections cured |
| | | | 500 mg (day 0 x 2) | 7/8 | 87.5% | 51.8% to 97.2% | | 96.3% (n = 27; 81.7 to 99.1%) CT infections cured |
| | | | 500 mg (day 0)<br>250 g (day 1 & 2) | 7/7 | 100% | NA | | 88.0% (n = 25; 69.8 to 95.6%) CT infections cured |
| Lassus (1990) [387] | Finland | NR | 1 (day 0) | 15/15 | 100% | NA | Culture and gram-stain for NG/CT w/ DFA; TOC day 14 | 100% (n = 12) CT infections cured<br>100% (n = 5) co-infections cured |
| | | | 500 mg (day 0)<br>250 mg (days 1 & 2) | 14/14 | 100% | NA | | 100% (n = 9) CT infections cured<br>83.3% (n = 6; 95% CI: 42.1 to 96.3%) co-infections cured |

TOC = Test of cure; NG = *Neisseria gonorrhoeae;* CT = *Chlamydia trachomatis;* LCR = Ligase chain reaction; DFA = direct fluorescent antibody; ELISA = Enzyme-linked immunosorbent assay; NR = Not reported ; NA = Not applicable

***In vitro* evidence**

Several *in vitro* studies have documented the loss of *N. gonorrhoea*e sensitivity to azithromycin over the past decade. There are no standard breakpoints of minimum inhibitory concentrations (MIC) used to categorize *N. gonorrhoeae* resistance to azithromycin, but $\geq$ 1 $\mu$g/mL and $\geq$ 2 $\mu$g/mL have both been used.[397] This section summarises key regional observations from 36 *in vitro* studies as shown in Tables 5.3b and 5.3c.

The Public Health Agency of Canada reported that 0.17% (N = 40,875; 95% CI: 0.001% to 0.002%) of *N. gonorrhoea*e samples were resistant to azithromycin between 2000 and 2009. The numeric mode of the MIC shifted from 0.25 $\mu$g/mL in 2001 to 0.5 $\mu$g/mL between 2007 and 2009.[398] During the same 10-year period, the Centers for Disease Control and Prevention in the United States reported that 0.04% (N = 87,566; 95% CI: 0.03% to 0.06%) of *N. gonorrhoea*e isolates tested had MICs ≥8 $\mu$g/mL (including 25 with 8 $\mu$g/mL and 14 with 16 $\mu$g/mL).[399] This did not include five cases of azithromycin resistance reported later that had been found between August and October 2009 amongst men who have sex with men. Three samples investigated had MICs of 8 $\mu$g/mL, whereas two had MICs of 16 $\mu$g/mL.[400]

## Table 5.3b Azithromycin and *Neisseria gonorrhoea*e: low-risk populations

| Reference (year of publication) | Country | Year(s) | Sample size | MIC range (μg/mL) | % strains susceptible | Additional details |
|---|---|---|---|---|---|---|
| Olsen (2013) [401] | Vietnam | 2011 | 108 | NR | 62% | 11% isolates fully resistant, 29% intermediate susceptibility |
| Lahra (2012) [402] | Australia | 2011 | 3,293 | ≤4 | 98.1% | Isolates from all states in Australia |
| Sethi (2013) [403] | India/Pakistan/Bhutan | 2007-2011 | 65 | 0.016 to 4.0 | 76.9% | 7.7% isolates fully resistant, 15.4% intermediate susceptibility |
| Lo (2012) [404] | Hong Kong | 2010 | 485 | <0.25 to >256 | 69.7% | -- |
| Lefebvre (2011) [405] | Canada | 2010 | 831 | ≤16 | 98.7% | -- |
| Hottes (2013) [406] | Canada | 2006-2011 | 1,837 | 0.064 to16 | 99% | Elevated MIC showed increasing trend over time |
| CDC (2011) [400] | United States | 2002-2009 | 87,566 | | 99.9% | 39 (0.04%) had MICs ≥8 μg/mL (25 with 8 μg/mL; 14 with 16 μg/mL) |
| Yuan (2011) [407] | China | 2008-2009 | 318 | NR | 94.7% | -- |
| Takahashi (2013) [408] | Japan | 2007-2009 | 52 | 0.016 to 1 | 100% | 100% of isolates from men[a] |
| Herchline (2010) [409] | United States | 2006-2008 | 286 | 0.032 to 1.0 | 99.0% | Median MIC 0.125 μg/mL |
| Cole (2010) [399] | Europe (17 nations) | 2006-2008 | 3,528 | ≤256 | 2.0% to 7.0% | High resistance (>256 μg/mL) in 4 isolates from Scotland and 1 in Ireland |
| Olsen (2012) [410] | Guinea-Bissau | 2006-2008 | 31 | NR | 100% | Of resistant strains, 2 had MIC >64 μg/mL |
| Tanaka (2011) [411] | Japan | 2001- 2009 | 242 | 0.004 to 1.0 | 99.9% | Modal shift of MIC was 0.25-0.5 μg/mL |
| Martin (2011) [398] | Canada | 2000-2009 | 40,875 | ≤64 | 99.8% | 100% isolates susceptible also to cefixime, ceftriaxone and spectinomycin. |
| Bala (2011) [412] | India | 2000-2009 | 274 | NR | 99.7% | One isolate was resistant to azithromycin, quinolones and penicillin |
| Chisholm (2009) [413] | United Kingdom | 2001-2007 | 108 | <0.03 to >256 | 94.0% | 6/108 isolates had MIC >256 μg/mL ; Shift to high level resistance |
| Khaki (2007) [414] | India | 2004-2005 | 60 | 0.016 to 0.25 | 100% | -- |
| Enders (2006) [415] | Southern Germany | 2004-2005 | 65 | 0.016 to 5.0 | 100% | 100% of isolates susceptible to azithromycin |
| Vorobieva (2007) [416] | Russia | 2004 | 76 | NR | 100% | Although all susceptible, reduced susceptibility seen in 14% |
| Sutrisna (2006) [417] | Indonesia | 2004 | 163 | NR | 100% | 53% resistant to ciprofloxacin |
| Martin (2004) [418] | Western Europe | 2004 | 965 | NR | 91.8% | 31.2% (Austria 30/96) to 0% (France 0/101, Greece 0/79, Portugal 0/17) |
| Chaudhary (2005) [419] | Nepal | 2003 | 16 | 0.008 to 0.5 | 100% | No isolates resistant, but 3/16 (19%) had reduced susceptibility |
| Chen (2009) [420] | Taiwan | 1999-2004 | 65 | NR | 100% | -- |
| Hsueh (2005) [421] | Taiwan | 1999-2003 | 55 | 0.03 to 9.0 | 72.7% | -- |
| Aydin (2005) [422] | Turkey | 1998-2002 | 78 | 0.004 to 0.25 | 100% | 100% of isolates from men[a] |
| Moodley (2001) [423] | South Africa | 1995-2000 | 58 | 0.015 to 1.0 | 100% | 37% (37/100) resistant to penicillin; tetracyclines reduced susceptibility. |
| Kobayashi (2003) [424] | Japan | 1995-1999 | 699 | 0.015 to 1 | 100% | 100% of isolates from men[a] |
| Llanes (2003) [425] | Cuba | 1995-1999 | 52 | 0.064 to 0.5 | 76.9% | 23.1% intermediate susceptibility: MIC= 0.125 (10 isolates) MIC= 0.5 (2 isolates) |
| Sosa (2003) [426] | Cuba | 1995-1999 | 91 | 0.063 to 4.0 | 90.1% | Isolates with reduced susceptibility also resistant to penicillin and tetracycline |
| Dillon (2001) [427] | Brazil | 1998 | 81 | 0.032 to 0.5 | 100% | 28% reduced susceptibility |
| Zarantonelli (1999) [428] | Uruguay | 1996-1997 | 51 | 0.032 to 0.5 | 100% | Decreased susceptibility (MIC 0.025 to 0.5) in 72%; isolates from men[a] |
| Young (1997) [429] | Scotland | 1996 | 67 | 0.023 to 0.75 | 100% | Isolates randomly selected from with penicillin MIC≥1 |
| Mehaffey (1996) [430] | United States | NR | 105 | 0.06 to 2.0 | 100% | Two tests compared. Data from agar dilution method not Etest. |
| Dillon (2001) | Guyana & St Vincent | 1992-1996 | 136 | 0.032 to 8.0 | 85.5% & 97.0% | 2 isolates had MIC =8ug/L. 49% (67/137) reduced susceptibility (combined) |
| Van Rijsoort-Vos (1995) [431] | Netherlands | 1991-1993 | 114 | 0.03 to 1.0 | 100% | 1 isolate reduced susceptibility |
| Ison (1993) [432] | South Africa | 1989-1990 | 192 | 0.03 to 1.0 | 100% | Study in migrant mine-workers (men only)[b] |

MIC = Minimum inhibitory concentration; NR = Not reported; CDC = Centers for Disease Control and Prevention ; [a]Sexual practices were not reported; isolates are assumed not to be from men who have sex with men; [b]Dislocated males workers are amongst high-risk populations;.

## Table 5.3c Azithromycin and *Neisseria gonorrhoeae*: high-risk populations

| Reference (year of publication) | Country | Year(s) | Sample size | MIC range (µg/mL) | % of strains susceptible | Additional details |
|---|---|---|---|---|---|---|
| **High-risk populations** | | | | | | |
| CDC (2011) [400] | United States | 2009 | 55 | NR | 90.9% | 9.1% resistant (95% CI: 4.0 to 19.6%); of 5 resistant (all from MSM), 3 had MIC equal to 8ug/mL and 2 had MIC equal to 16ug/ml |
| Starnino (2009) [433] | Italy | 2007-2008 | 219 | 1.0 to 256.0 | 90.0% | 72.7% (95% CI: 51.6 to 86.8%) of resistant isolates from MSM |
| Donegan (2006) [434] | Bali | 2004 | 147 | 0.013 to 0.512 | 100% | Study in FSWs; prevalence of NG estimated to be 35-60% |
| Morris (2009) [435] | United States | 2000-2002 | 79 | 0.03 to 0.5 | 100% | Increased MIC values seen in MSM subject isolates |
| Ieven (2003) [436] | Indonesia | 1996 | 267 | 0.032 to 0.5 | 100% | Study in FSWs; prevalence of NG estimated to be 18-44% |
| CDC (2000) [437] | United States | 1999 | 12 | 1.0 to 4.0 | NR | Median MIC was 2.0ug/mL. 6 of 12 samples were from men who had sex with a CSW; 2 of 12 were from HIV positive men |
| **Mixed-risk populations** | | | | | | |
| Bruck (2012) [438] | United Kingdom | 2005-2006 | 147 | NR | 99.3% | Mixed male heterosexual, MSM and female heterosexual isolates |
| Dan (2010) [439] | Israel | 2002-2007 | 406 | 0.023 to 8.0 | 91.8% | Mixed male heterosexual, MSM and female heterosexual; resistance to azithromycin did not appear to rise over 5 year period |
| McLean (2004) [440] | United States | 1999-2001 | 1,248 | ≤4 | 97.4% | Mixed high risk and low risk population. Median MIC was 2.0ug.mL |
| Arreaza (2003) [441] | Spain | 1992-2001 | 63 | 0.03 to 4.0 | 96.8% | 58.7% of strains had reduced susceptibility (0.25-1.0ug/ml). 50% of resistant isolates were from FSW |

* Mixed-risk population; MIC = Minimum inhibitory concentration; NR = Not reported; MSM = Men who have sex with men; FSWs = Female sex workers; NG = *Neisseria gonorrhoeae*; CSW = Commercial sex worker (gender not specified); CDC = Centers for Disease Control and Prevention

Gonococcal resistance may have appeared in Europe slightly before North America. Analysis of isolates from 17 European countries suggests that 3.2% (N = 836; 95% CI: 0.02% to 0.05%) of gonococcal isolates were resistant to azithromycin in 2006. By 2007, 6.8% (N = 973; 95% CI: 0.05 to 0.09) of samples were resistant. Sampling in 2008 found 1.8% (N = 940; 95% CI: 0.01% to 0.03%) of isolates to be resistant. However, only 5.2% (95% CI: 4.1% to 6.8%) of strains tested in the same year were fully susceptible to azithromycin and ciprofloxacin. Four isolates from Scotland and one from Ireland exhibited MICs >256 mg/l.[399]

Gonococcal isolates from South America and Cuba exhibited high but stable levels of resistance between 2000 and 2009 in most settings.[442] Collectively, azithromycin resistance was 13.0% (N = 8,373; 95% CI: 12.3% to 13.7%) based on data from six countries including Chile, an outlier. Averaged over the decade, 26.7% (N = 3,116; 95% CI: 25.2% to 28.3%) of samples from Chile were resistant, rising to 45.6% (N = 463; 95% CI: 41.1% to 50.1%) in the most recent data from 2009. Meta-analysis using random effects models, excluding data from Chile, suggests that only 4.4% (N = 5,257; 95% CI: 3.9% to 5.1%) of isolates were resistant over the decade.

All 60 gonococcal isolates from India between 2004 and 2005 were susceptible to azithromycin.[414] Published analysis of samples collected from India, Pakistan and Bhutan between 2007 and 2011 found that 76.9% (N = 65; 95% CI: 65.3% to 85.5%) were susceptible. Results were not stratified by country and, therefore, it is unknown whether the sensitivity of isolates from India had changed during the same period.[403] Applying the more conservative breakpoint of $\geq 1$ $\mu$g/mL to the *in vitro* studies

identified in this systematic review, 35% (7 of 20) of the *in vitro* studies reported upper-range MICs that included gonococcal isolates resistant to azithromycin. This percentage does not include 16 studies identified and included in Tables 5.3b and 5.3c that did not report MICs.

### *Chlamydia trachomatis* and azithromycin

#### *In vivo* evidence

The WHO recommends treating pregnant women with *C. trachomatis* infection using 1 g azithromycin as a single oral dose.[110] There were eight RCTs in the literature that reported the treatment efficacy of 1 g azithromycin amongst pregnant women [443-450] as summarised in Table 5.4a. Meta-analysis using random effect models suggests the pooled treatment efficacy was 92.1% (N = 268; 95% CI: 88.4% to 95.7%). The estimated efficacy would be higher if two trials from the United States had been excluded. The first trial (1995 to 1997) reported a three-week test of cure rate to be 88.1% (N = 42; 95% CI: 74.9% to 94.7%).[446] The second trial (1998 to 2000) was terminated early, however, as tests of cure conducted four or more weeks post-treatment showed that only 63.3% of infections had been cleared (N = 55; 95% CI: 50.4% to 75.1%).[444] These results need to be interpreted with caution because no distinction was made between treatment failures and new infections. Moreover, sex partners were referred to treatment centres, and only 35% of women were seen by trial staff within seven days of the scheduled test of cure.

## Table 5.4a Azithromycin and *Chlamydia trachomatis* in pregnant women

| Reference (year of publication) | Country | Year(s) | No. cured of evaluated | Cured (%) | 95% CI | Diagnostic method | Birth outcomes | Additional details |
|---|---|---|---|---|---|---|---|---|
| Kacmar (2001) [443] | United States | 1998-2000 | 18/19 | 94.7% | 84.4% to 100%* | Ligase chain reaction; TOC 28-42 days | NR | 52.6% (n = 19; 95% CI: 31.5% to 72.8%) had side effects; passive or active solicitation not reported; 13.6 weeks (±8.0 SD) mean gestational age at enrolment |
| Jacobson (2001) [444] | United States | 1998-2000 | 35/55 | 63.6% | 47.7% to 79.6% | DNA LCx STD assay; TOC 28 days | 13.3% (6/45) preterm | 10.1% (n = 55; 95% CI: 5.2% to 21.9%) had side effects; passive or active solicitation not reported; 20.6 weeks (± 8.8 SD) mean gestational age at enrolment |
| Wehbeh (1998) [445] | United States | NR | 26/27 | 96.3% | 89.0% to 100%* | Culture | NR | 100% (n=27) of partners treated |
| Adair (1998) [446] | United States | 1995-1997 | 37/42 | 88.1% | 77.7% to 98.5% | DNA assay; TOC 28 days | NR | 11.9% (n = 5; 95% CI: 5.3% to 25.1%) had side effects; passive or active solicitation was not reported; 21.6 weeks (±9.5 SD) mean gestational age at enrolment; 54.8% (n = 23) of partners treated |
| Edwards (1996) [448] | United States | 1993-1994 | 61/65 | 93.8% | 87.8% to 99.9% | DNA assay; TOC 14 days | 9.2% (6/65) preterm, 3 due to PROM; Mean gestational age at delivery 38.8 wks ±1.6 | 20.4 weeks mean gestational age at enrolment |
| Rosenn (1995) [449] | United States | 1994-1995 | 21/23 | 91.3% | 79.3% to 100%* | PCR; TOC 21 days | NR | 19.3 weeks (± 3.5 SD) mean gestational age at enrolment |
| Gunter (1996) [447] | United States | NR | 22/22 | 100.0% | NA | DNA assay; TOC 14 days | NR | 13.6% (n = 3; 95% CI: 5.0% to 33.6%) had gastrointestinal side effects; passive or active solicitation not reported |
| Bush and Rosa (1994) [450] | United States | NR | 15/15 | 100.% | NA | DNA assay; TOC 14 days | NR | 0% of women experienced side effects; 100% (n = 15) of partners treated |
| Rahangdale (2006) [451] | United States | 1999-2000 | 137/141 | 97.2% | 94.4% to 99.9% | DNA assay; TOC days 7-20; 21-34; 35-55; >56 | 7.5% (16/221 'any' preterm azithromycin) | 13.1% (n = 191; 95% CI: 9.0% to 18.6%) also had BV 14.1 weeks (± 6.3 SD) mean gestational age at enrolment |
| Miller (1995) [452] | United States | 1993-1994 | 132/138 | 95.6% | 92.2% to 99.1% | DNA assay; TOC 10-14 days | 15.2% (19/125) preterm | 5.5% (n = 146; 95% CI: 2.8% to 10.4%) had side effects; 23.9% (n = 138; 17.6% to 31.7%) women also had NG; 17.4% (4/17) reported side effects (all GI); mean gestational age at enrolment not reported |

Note that all studies used 1 g of azithromycin delivered as 1 dose; CI = Confidence interval; TOC = Test of cure; NR = Not reported; DNA = Deoxyribonucleic acid; PROM = Preterm premature rupture of the membranes; SD = Standard deviation; NG = *Neisseria gonorrhoeae*; BV = Bacterial vaginosis; GI = gastrointestinal; Upper confidence interval exceeded 100% and, therefore, was cut-off.

Studies investigating sexual activity following treatment may help to explain post-treatment infections; misclassification of treatment failures and *de novo* infections may have been at play. A trial in Seattle (1998 to 2003) found that persistent or recurrent chlamydial or gonorrhoeal infection occurred in 7.6% (N = 289; 95% CI: 5.1% to 4.9%) of female patients who reported no sexual intercourse after treatment.[453] Another study reported that 19.0% (N = 79; 95% CI: 11.9% to 29.0%) of women were positive for *C. trachomatis* three months after treatment using 1 g azithromycin. Of these women, 13.3% (N = 15; 95% CI: 4.0% to 38.3%) reported being inactive sexually during the post-treatment period.[454] These findings may be attributable to: (i) false reporting of sexual contact, (ii) treatment failure, or (iii) may lend credence to the hypothesis that *C. trachomatis* enters a latent asymptomatic state which is undetectable by culture or, possibly, Nucleic Acid Amplification Tests for a time-limited period and can later reactivate.[455]

***In vitro* evidence**

Specific thresholds for antimicrobial susceptibility and resistance of *C. trachomatis* are not universally standard, although MICs of > 4 μg/ml (microgram per millilitre) are often used to characterize therapeutic failure.[456-459] The lowest concentration of antimicrobial needed to inhibit chlamydial development is between 0.03 and 0.125μg/ml, whereas the minimum bactericidal concentration (MBC; also referred to as the minimum chlamydicidal concentration, or MCC), is between 0.06 and 0.5μg/ml.[460,461] Current evidence from *in vitro* studies suggest that azithromycin maintains high and widespread treatment efficacy against *C. trachomatis* as summarised in Table 5.4b. One exception, however, is a study of six isolates from

three patients who experienced treatment failure in Russia (2000 to 2002). Four isolates were resistant to azithromycin, doxycycline and ofloxacin at MICs and MBCs > 5.12µg/ml.[462] Not surprisingly, *in vitro* resistance appears to be more common amongst isolates from individuals with greater severity of disease or recurrent disease.[463] A study in the United States during the early 1990s described decreased susceptibility and emerging resistance to azithromycin and doxycycline in isolates from women with mucopurulent cervicitis, whereas isolates from women with asymptomatic infections remained susceptible.[464]

## Table 5.4b Azithromycin and *Chlamydia trachomatis*: low-risk populations

| Reference (year of publication) | Country | Year(s) | Azithromycin | | Other macrolides | | Additional details |
|---|---|---|---|---|---|---|---|
| | | | MIC (µg/ml) | MBC/MCC (µg/ml) | MIC (µg/ml) | MBC (µg/ml) | |
| **No resistance observed** | | | | | | | |
| Ljubin-Sternak (2012) [465] | Croatia | 2010 | 0.064 to 0.125 | 0.064 to 2.0 | Doxycycline: 0.016 to 0.064 | 0.032 to 1.0 | 24 urogenital strains assessed |
| Donati (2010) [466] | Italy | 2005-2006 | 0.25 to 0.5 | 0.5 to 1.0 | Doxycycline: 0.03 to 0.06<br>Erythromycin: 0.5 to 1.0 | 0.06 to 0.125<br>1.0 to 2.0 | All serovars had comparable susceptibilities.<br>Azithromycin and doxycycline bactericidal with MBC at 1-2 times the MIC. (50 strains) |
| Hong (2009) [467] | Ethiopia | 2006 | 0.25 to 0.5 | 0.25 to 1.0 | Doxycycline: 0.015 to 0.03 | 0.03 to 0.06 | Azithromycin unchanged between pre- and post-mass biannual treatment of trachoma (10 strains) |
| Samra (2001) [459] | Israel | 1997-1999 | 0.06 to 0.125 | 0.06 to 0.25 | Doxycycline: 0.125 to 0.25<br>Tetracyclines: 0.25 to 0.5 | 0.125 to 4.0<br>0.25 to 4.0 | Smallest MBC and MIC difference in azithromycin vs. doxycycline (4 dilutions differences; 50 isolates) |
| Lefèvre (1993) [468] | France | NR | 0.06 to 0.125 | 0.25 to 0.5 | Clarithromycin: 0.008<br>Erythromycin : 0.06 to 0.125<br>Tetracyclines: 0.125 to 0.25 | 0.03 to 0.125<br>0.25 to 2.0<br>1.0 to 4.0 | 15 clinical isolates tested |
| Agacfidan (1993) [469] | United States | 1993 | ≤0.06 to 1 | 0.12 to 2.0 | Doxycycline: 0.015 to 0.06<br>Tetracyclines: 0.03 to 0.12 | 0.015 to 0.06<br>0.06 to 0.12 | Azithromycin highly active against CT in isolates from urethral and cervical samples (azithromycin 10 strains, doxycycline 22 strains, tetracycline 22 strains) |
| Scieux (1990) [470] | United Kingdom | 1990 | 0.064 to 0.25 | 2.0 to 8.0 | Doxycycline: 0.016 to 0.064<br>Erythromycin: 0.064 to 0.128 | 0.5 to 8.0<br>0 to 64.0 | 10 strains used from the United States |
| **Resistance observed** | | | | | | | |
| Bhengraj (2010) [464] | India | 2006-2007 | 0.12 to 8 | ≤ 8.0 | Doxycycline: 0.025 to 8.0) | ≤8.0 | Decreased antimicrobial susceptibility in recurrently infected female patients (21 isolates) |
| Misyurina (2004) [462] | Russia | 2000-2002 | >5.12 | > 5.12 | Erythromycin: > 5.12 | >5.12 | Isolates from salpingitis, endocervicitis, and urethritis showed resistance (6 isolates) |
| Somani (2000) [458] | United States | 1995-1998 | 0.5 to >4.0 | > 4.0 | Doxycycline: 0.125 to > 4.0 | >4.0 | Resistance of strains causing relapsing or persistent infection in 3 patients (3 isolates) |
| Rice (1995) [463] | United States | 1995 | 0.125 to 2.0 | 0.5 to > 4.0 | Doxycycline: 0.008 to 0.06 | 0.015 to 4.0 | Isolates susceptible to azithromycin and doxycycline in asymptotic infection |

MIC = Minimum inhibitory concentrations; MBC = Minimum bactericidal concentration; MCC = Minimum chlamydicidal concentrations; NR = Not reported; CT = *C. trachomatis*; PID = Pelvic inflammatory disease

***Trichomonas vaginalis***
***In vivo* evidence**

*Trichomonas vaginalis* is a protozoal infection that produces cervicitis and non-gonococcal urethritis. Treatment of pregnant women with *T. vaginalis* infection after the first trimester, per WHO recommendations, requires the administration of 2g metronidazole as a single dose, or 400-500 mg twice daily for seven days, or 300 mg clindamycin orally twice a day for seven days.[110] If treatment is imperative during the first trimester of pregnancy, the single-dose regimen of 2 g metronidazole orally is recommended.[110] Azithromycin has not been used directly for prevention or treatment purposes because *T. vaginalis* is anaerobic. Nevertheless, azithromycin has demonstrated prophylactic protection against *T. vaginalis* in studies of mass STI/RTI treatment as summarised in Tables 5.5a and 5.5b.

In Kenya (1998 to 2002), 1 g azithromycin was compared to placebo and administered once per month to 466 HIV-negative female sex workers.[471] At the end of the trial, HIV incidence was the same across treatment groups, the primary endpoint, but the risk of *T. vaginalis* was reduced by one-half amongst female sex workers who were given azithromycin versus placebo (RR = 0.56; 96% CI, 0.40 to 0.78; $P < 0.001$).

## Table 5.5a RCTs of azithromycin combinations amongst pregnant women

| Reference (year of publication) | Country | Year(s) | Regimen | Number of cases post treatment (%) | | | | | Additional details |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Treponema pallidum** | *Neisseria gonorrhoeae* | *Chlamydia trachomatis* | *Trichomonas vaginalis* | Bacterial vaginosis | |
| Luntamo (2010) [107] | Malawi | 2003-2006 | **Intervention** 1 g AZ x 2 + SP monthly | NR | 0.5% (2/391) | 0.3% (1/391) | 11.0% (46/419) | NR | Significant protection against *T. vaginalis* $P = 0.05$ |
| | | | **Intervention** SP monthly | NR | 2.1% (8/384) | 0.3% (1/384) | 15.1% (62/411) | NR | |
| | | | **Control** SP x 2 | NR | 0.7% (3/391) | 0.3% (1/391) | 16.7% (69/411) | NR | |
| van den Broek (2009) [108] | Malawi | 2004-2005 | **Intervention** 1 g AZ x 2 + SP x2 | NR | NR | NR | NR | NR | No difference in preterm birth (16.8% versus 17.4%); potential explanatory factors include use of sub-optimal syphilis treatment [2,472] |
| | | | **Control** SP x 2 | NR | NR | NR | NR | NR | |
| Gray (2001) [358] | Uganda | 1994-1998 | **Intervention** 1 g AZ + 250 mg CIPX + 2g MTZ | 3.4% (57/1,677) | 0.9% (14/1,503) | 1.1% (16/1,503) | 4.7% (4/1,779) | 36.3% (645/1,779) | Neonatal death RR=0.83; 95% CI: 0.71 to 0.97; Low birth weight RR=0.68; 95% CI: 0.53 to 0.86; Preterm delivery RR=0.77; 95% CI: 0.56 to 1.05. Vertical transmission of HIV was no different between intervention and control groups. |
| | | | **Control** Iron-folate + 100 mg MBZ x2 | 3.3% (46/1,376) | 1.7% (24/1,394) | 2.7% (38/1,394) | 15.9% (248/1,569) | 48.5% (764/1,576) | |
| Wawer (1999) [473] | Uganda | 1994-1998 | **Intervention** 1 g AZ + 250 mg CIPX + 2 g MTZ | 6.0% (80/1,323) | 1.0% (8/770) | 1.2% (9/770) | 5.3% (72/1,350) | 39.1% (533/1,364) | Vertical transmission of HIV was no different between intervention and control groups. |
| | | | **Control** Iron-folate + 100 mg MBZ x2 | 7.1% (75/1,056) | 2.1% (15/714) | 3.5% (25/714) | 17.4% (198/1,137) | 52.8% (609/1,154) | |

*2.4mμ benzathine Penicillin G was administered to pregnant women in all treatment groups per national guidelines (exception being [108]); AZ = Azithromycin; SP = Sulphadoxine-pyrimethamine; NR = Not reported; RR = Risk ratio; CI = Confidence interval; CIPX = Ciprofloxacin; HIV = Human immunodeficiency virus

## Table 5.5b RCTs of azithromycin combinations amongst commercial sex workers

| Reference (year of publication) | Country | Year(s) | Regimen | | Proportion of cases cured (cases pre-treatment / cases post-treatment) | | | | | Additional details |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Syphilis* | *N. gonorrhoeae* | *C. trachomatis* | *T. vaginalis* | Bacterial vaginosis | |
| Kaul (2004) [471] | Kenya | 1998-2002 | **Intervention** 1 g AZ monthly (multi-yr pd) | | 3.9% | 2.6% | 1.1% | 11.3% | 53.0% | Incidence reported per 100 women-years |
| | | | **Control** Placebo | | 3.8% | 5.7% | 6.5% | 20.4% | 57.4% | |
| Labbe (2012) [474] | Benin Ghana | 2001 | **Intervention** 1 g AZ or 500 g CIPX monthly for 9 mo alternating AZ & CIPX | | NR | 5.6% (7/126) | 1.6% (2/126) | NR | NR | Significant protection against NG *P* = 0.05 |
| | | | **Control** Placebo | | NR | 12.5% (14/112) | 2.7% (3/112) | NR | NR | |
| Cowan (2005) [475] | Zimbabwe | NR | **Intervention** 1 g AZ + 2 g MTZ + 500 mg CIPX | | Base: 5.0% (2.8, 8.7%); *V2, V3, V4* = NR in any form | Base: 1.9% (0.5, 3.4%); graphs contain visible inaccuracies for *V2, V3, V4* | Base: 1.7% (0.3, 3.0%); graphs contain visible inaccuracies for *V2, V3, V4* | Base: 19.3% (15.2, 23.4%) *V2: 4.3% (7.7 to 2.2%)* *V3: 12.6% (17.7 to 8.8%)* *V4: 11.5% (15.7 to 7.8%)* | -- | -- |
| | | | **Intervention** 1 g AZ + 2 g MTZ | | | | | | | |
| Wi (2006) [476] | Philippines | 2001 | **Intervention** 1 g AZ one time | Prior to intervention | NR | 18.3% (207/1,130) | 28.6% (323/1,130) | NR | NR | -- |
| | | | | 1 mo post-intervention | | 11.9% (82/687) | 15.1% (104/687) | | | |
| Williams (2003) [477] | South Africa | 1998-2000 | **Intervention** 1 g AZ 9x in 9 mo | Prior to intervention | 9.8% (68/691) | 6.9% (48/691) | 7.9% (55/691) | NR | NR | HIV prevalence amongst CSW in the mining community was 68.6% |
| | | | | 9 mo post-intervention | 18.7% (166/893) | 8.6% (77/893) | 13.8% (123/893) | | | |
| Steen (2000) [478] | South Africa | 1996-1997 | **Intervention** 1 g AZ every mo for 9 mo | Prior to intervention | NR | 17.3% (70/407) | 14.3% (58/407) | NR | NR | Pre-intervention NG and/or CT = 24.9% (101/407); Post-intervention NG and/or CT = 5.7% (6/108) |
| | | | | 9 mo post-intervention | | 4.7% (5/108) | 0.9% (1/108) | | | |

Note that none of the azithromycin combinations summarised are contraindicated in pregnancy

*2.4 mμ benzathine Penicillin G was administered to commercial sex workers who tested positive for syphilis in all treatment groups per national guidelines; AZ = Azithromycin; NR = Not reported; CIPX = Ciprofloxacin; MTZ = Metronidazole; HIV = Human immunodeficiency virus; Note that italicized values are approximate based on enlarged graphs published in Cowan *et al* and percentages in parentheses reflect the 95% confidence intervals [475]; *V2, V3, V4* = Visit 2, Visit 3, and Visit 4; HIV = Human immunodeficiency virus; NG = *Neisseria gonorrhoeae*; CT = *C. trachomatis*; CSW = Commercial sex worker

A similar observation was made in a three-arm IPTp trial conducted in Malawi (2003 to 2006) in a low-risk population of pregnant women.[107] Participants received standard IPTp-SP, or monthly IPTp-SP, or monthly IPTp-SP plus 1 g azithromycin during two antenatal visits. The prevalence of *T. vaginalis* at delivery was 16.7% (n = 411; 13.5% to 20.7%), 15.1% (n = 411; 95% CI: 12.0% to 18.9%), and 11.0% (n = 419; 95% CI: 8.3% to 14.3%), respectively. Thus, compared to recipients of monthly IPTp-SP alone, women who received monthly azithromycin plus IPTp-SP had 35% (RR = 0.65; 0.46 to 0.93; *P* = 0.02) fewer *T. vaginalis* infections at delivery.

A cluster randomised trial in Uganda (1994 to 1998) compared the incidence of HIV infections amongst non-pregnant adults who received three antibiotics (1 g azithromycin, 250 mg ciprofloxacin, and 2 g metronidazole) or multi-vitamins plus antihelminth treatment.[473] Although the trial was terminated early for lack of protection against the primary endpoint (incidence of vertical transmission of HIV infection), the incidence of several curable STIs/RTIs was lower in the control group, most notably *T. vaginalis*. The cumulative incidence of newly diagnosed *T. vaginalis* infection was 4.8 per 100 person years (116/2397 person-years) in the intervention group, compared to 9.1 per 100 person-years (182/1993 person-years) in the control group (RR=0.52; 0.35 to 0.79). The same combination of antimicrobials (azithromycin, ciprofloxacin, and metronidazole) was provided to female sex workers in rural Zimbabwe as a one-time treatment followed by three monthly check-ups.[475] The prevalence of *T. vaginalis* pre-dose was just under 20% and then decreased to approximately 5% at visit 2. It rose to nearly 13% at visit 3, and dropped again to just over 10%, one-half of the pre-treatment levels.

**Bacterial vaginosis**

The WHO recommendations are to treat bacterial vaginosis in pregnant women, preferably after the first trimester, using: (i) 200 or 250 mg metronidazole three times per day for seven days, or (ii) 5 g metronidazole gel (0.75%) applied intravaginally twice a day for five days, or (iii) 300 mg clindamycin 300 mg orally twice a day for seven days.[109] As with *T. vaginalis*, 2g metronidazole orally is recommended if treatment is imperative during the first trimester of pregnancy.[109] Bacterial vaginosis has no single causative agent, but is thought to result from destabilization of *Lactobacillus* species (spp.) which leads to secondary colonization of anaerobic organisms that include *Gardnerella vaginalis, Bacteroides* spp.*, Mobiluncus* spp. and *Mycoplasma hominis.* These changes in flora accompany an increase in vaginal pH.[479,480]

***In vivo* evidence**

There were no trials identified by systematic review that attempted to measure the treatment efficacy of azithromycin alone against bacterial vaginosis. However, one study in the United States (2002 to 2005) investigated the use of azithromycin as a partner drug with metronidazole for the treatment of symptomatic bacterial vaginosis. Non-pregnant women were randomised into four treatment groups and received 750 mg metronidazole once per day for 7 days, or metronidazole once per day for 7 days plus 1 g azithromycin on days 1 and 3, or metronidazole for 14 days, or metronidazole for 14 days plus azithromycin on days 1 and 3.[481] There was no additional benefit of cure observed amongst women who received metronidazole plus azithromycin compared to metronidazole alone. Because bacterial vaginosis is a syndrome that involves multiple micro-organisms, antibiotic treatment is challenging. However,

comparable data from other related macrolides do suggest potential therapeutic benefit for azithromycin in the treatment of bacterial vaginosis as shown in Table 5.6 Analysis of azithromycin against the anaerobic and carboxyphilic bacteria which replace the normal vaginal flora may provide a better understanding as to the potential role of azithromycin against bacterial vaginosis.

## Table 5.6 Macrolides and isolates of key organisms in bacterial vaginosis

*Unspecified low risk population*

| | Reference (year of publication) | Country | Year(s) | Minimum inhibitory concentrations of specific macrolides (µg/mL) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Azithromycin | Erythromycin | Clarithromycin | Roxithromycin | Clindamycin* | Telithromycin* |
| ***Gardnerella vaginalis*** | Jones (1988) [482] | United Kingdom | NR | < 0.03 to 0.125 | < 0.03 to 0.06 | NR | NR | NR | NR |
| | Shanker (1982) [483] | Australia | NR | NR | 0.007 to 0.06 | NR | NR | 0.007 to 0.06 | NR |
| | Ridgway (1987) [484] * | | NR | NR | 0.008 to 0.016 | NR | 0.016 | NR | NR |
| ***Bacteroides species*** | Jones (1998) [482] | United Kingdom | NR | 0.06 to16 | < 0.03 to 32 | NR | NR | NR | NR |
| | Dubreuil (1987) [485] | England, France, Germany, Japan | NR | NR | 0.003 to > 64 | NR | 0.003 to > 64 | NR | NR |
| | Maskell (1990) [486] | United Kingdom | NR | 0.5 to > 16 | < 0.25 to 16 | NR | NR | NR | NR |
| | Chang (1995) [487] | Taiwan | 1989-1992 | 1 to > 256 | 0.25 to > 256 | ≤0.03 to > 256 | 0.25 to > 256 | NR | NR |
| | Ednie (1997) [488] | United States | NR | 1 to > 64 | 0.5 to > 64 | 0.5 to > 64 | 2 to > 64 | ≤ 0.06 to > 64 | NR |
| | Mikamo (2003) [489] | Japan | 2000 | 0.125 to 32 | 0.125 to 32 | 0.063 to16 | NR | NR | 0.032 to 16 |
| | Marina (2009) [490] | Bulgaria | 1983-2007 | NR | 0.5 to > 64 | NR | NR | 0.125 to 32 | NR |
| | Chen (1992) [491] | Australia | 1986-1991 | 0.5 to 128 | 0.25 to 128 | NR | NR | NR | NR |
| | Wexler (2001) [492] | United States | NR | NR | NR | NR | NR | NR | 0.25 to > 64 |
| ***Mycoplasma hominis*** | Ridgway (1987) [484] | United Kingdom | NR | NR | >32 | NR | 8 to16 | NR | NR |
| ***Mobinculus species*** | Spiegel (1987) [493] | United States | NR | NR | ≤0.2 to > 200 | NR | NR | ≤ 0.015 to 4 | NR |

*Not a macrolide but has similar mechanism of action and included for comparability; NR=Not reported

## Discussion

This systematic review of *in vivo* efficacy and *in vitro* sensitivity of azithromycin against curable STIs/RTIs suggests the compound is an attractive option for preventing and curing *T. pallidum, N. gonorrhoeae* and *C. trachomatis* infections. Prior to the advent of antiretroviral therapies for HIV, the management of curable STIs/RTIs received notable importance as trials showed that treatment of *N. gonorrhoeae, C. trachomatis,* and *T. vaginalis* reduced the genital viral load of HIV amongst men and women.[494-497] Thus, groups at high-risk for transmitting HIV have since been targeted by treatment campaigns using 1 g azithromycin. It is not surprising, therefore, that changes in azithromycin sensitivity in high-income settings have often been observed first amongst members of high-risk groups who may have had compromised immunity attributable to HIV infection. Pregnant women attending ANC facilities in sub-Saharan Africa do not share this risk profile. Thus, on this basis alone, it is less likely that the use of azithromycin in combination with an antimalarial compound, would be a catalyst for the rapid selection of azithromycin resistance, although such a potential cannot be ruled out.

The potential benefits of using azithromycin as part of an enhanced IPTp regimen may be best viewed through prior experience with mass drug administration amongst pregnant women as noted in the prior section on *T. vaginalis*. In the context of the AIDS epidemic and before the age of antiretroviral therapies, investigators attempted to prevent vertical transmission of HIV by providing pregnant women in Uganda 1 g azithromycin, in combination with 250 mg ciprofloxacin and 2 g metronidazole.[358] The data safety monitoring board suspended the trial early for reasons of futility, despite

having cut neonatal deaths by 17% (RR = 0.83; 95% CI: 0.71 to 0.97), decreased the incidence of LBW by 32% (RR = 0.68; 95% CI: 0.53 to 0.86), and reduced the incidence of preterm delivery by 23% (RR = 0.77; 95% CI: 0.56 to 1.05). These impressive results were achieved at a time when neither IPTp-SP nor insecticide treated bed nets for the control of malaria in pregnancy had been deployed.

If azithromycin is provided as part of IPTp alongside a partner antimalarial drug, there are several key factors to consider that are pathogen specific. Regarding syphilis, no less than 1 g azithromycin should be used alongside 2.4 mμ BPG for three reasons: (i) combination therapy has been shown to achieve higher rates of cure than either therapy alone,[498] (ii) use of azithromycin with BPG would likely reduce selection of the A→G mutation associated with azithromycin and preserve *T. pallidum* sensitivity, and (iii) most importantly, only BPG can be expected to cure congenital infection if the placenta has been invaded by spirochetes.[111]

As for *N. gonorrhoeae*, 1 g azithromycin may be just above the minimum inhibitory concentration of fully susceptible strains and, therefore, more than 1 g azithromycin may be preferable from the standpoint of reducing selection pressure. However, a single 2 g dose may not be well tolerated as six in ten patients reported self-limiting gastrointestinal discomfort when treated for syphilis infection with such a regimen. Splitting the dose over two days may improve tolerability. This is an important issue based on the experience of azithromycin plus chloroquine in which 1 g azithromycin was given on three consecutive days. Treatment-related adverse events were experienced by nearly seven of ten women, 68.9% in total (n = 996). The most

frequent events were vomiting, dizziness, headache, and asthenia amongst 44.6%, 31.4%, 15.3%, and 15.2% of azithromcycin plus chloroquine recipients, respectively.[116] If the partner antimalarial drug is dihydroartemisinin-piperaquine, therapy that requires a three-day course, a split dose of 500 mg might be considered for each of the three days to achieve optimal tolerability. This would also simplify dosing, and likely improve compliance and adherence to the regimen.

A regimen of 1 g azithromycin, would be protective against *C. trachomatis* and, although the data are limited and the mechanism of action is not understood, 1 g azithromycin may protect against *T. vaginalis* based on reports from Malawi amongst pregnant women[107] and commercial sex workers in Kenya.[471] It is curious, however, that *T. vaginalis* infection during pregnancy is associated with adverse birth outcomes, but the first-line treatment of 2 g metronidazole does not always improve birth outcomes. A trial in Uganda reported that pregnant women treated for *T. vaginalis* infection were 2.5 times more likely to deliver a LBW infant than untreated women (RR = 2.49; 95% CI: 1.12 to 5.50).[499] The authors suggest this may be attributable to metronidazole exposure. Another trial in the United States reported an increase in the risk of preterm delivery amongst pregnant women exposed to metronidazole for the treatment of asymptomatic trichomoniasis compared to those who were not treated (RR = 1.8; 95% CI: 1.2 to 2.7)[500]. In contrast to these findings from a high-income setting, data from a multi-centre trial in sub-Saharan Africa suggests that treatment of *T. vaginalis* infection using metronidazole does not increase the chances of preterm birth.[501] Apart from bacterial vaginosis, which is not transmitted through sexual

contact, re-infection will remain a risk for pregnant women and, therefore, providers should continue to offer education and screening as appropriate.

None of the studies identified in this review indicate that azithromycin offers preventive or curative effect against bacterial vaginosis, the most prevalent of curable STIs/RTIs. Antibiotic therapy with metronidazole has only been shown to reduce the risk of preterm delivery by one-half (RR = 0.53; 95% CI: 0.34 to 0.84) amongst pregnant women with bacterial vaginosis (Nugent score 7 to 10) or intermediate flora (Nugent score 4 to 6).[502] A Nugent score of 0 to 3 is considered normal,[503] for which no protection against adverse birth outcomes has been observed.

## Summary discussion

Since the WHO first recommended IPTp in 2004 for pregnant women resident in malaria endemic areas during antenatal visits, clinical researchers have been trying to identify replacements for SP.[116,154,504-506] These efforts have proven elusive in large part because IPTp-SP has only been viewed as intervention that reduces the consequences of malaria infection on pregnancy outcomes. This has led investigators to assume that there must be a threshold of malaria transmission intensity in sub-Saharan at the population level below which IPTp-SP no longer protects against the incidence of LBW. There does not appear to be one based on evidence presented in this thesis as well as unpublished findings from analysis of Multiple Indicator Cluster Surveys (Eisele TP, personal communication). Evidence presented in this thesis does suggest that the protective effect of IPTp-SP against LBW is under threat by a higher prevalence of the biomarker A581G associated with SP resistance. However $\geq$ 2 doses of IPTp continues to protect primi- and secundigravidae against the incidence of LBW in areas where A581G can be found in over one-half of malaria parasites.

This protection could extend to higher prevalence levels, but there are no data available to support or refute this assertion. However, in areas with a prevalence of the A581G mutation > 10.1%, IPTp-SP may not have a beneficial effect on LBW. This observation needs to be interpreted with caution because there were no data in areas with a prevalence between 10% and 50%.

IPTp-SP continues to proect against the incidence of LBW across all malaria transmission intensities, as well as in the presence of the A581G mutation, particularly amongst primi- and secundigravidae, because the causes of LBW are multifactorial. This has long-been assumed, but the secondary analysis of data from Zambia in this thesis is the first evidence that IPTp-SP is protective against adverse birth outcomes related to curable STIs/RTIs.

The systematic review and meta-analysis of the dual burden of malaria and curable STIs/RTIs produced the first published pooled estimates of peripheral (N = 90,755; 94 studies; 16 countries) and placental (N = 34,184; 35 studies; 13 countries) malaria infection amongst women attending ANC facilities in sub-Saharan Africa. The pooled estimates fall within the range of point estimates contained in prior reviews by Brabin and Steketee *et al*. This was also the first such meta-analysis of curable STIs/RTIs in the region, producing pooled estimates for syphilis (N = 147,477; 50 studies; 18 countries), *N. gonorrhoeae* (N = 19,957; 19 studies; 12 countries), *C. trachomatis* (N = 10,573; 22 studies; 15 countries), *T. vaginalis* (N = 37,995; 35 studies; 15 countries), and bacterial vaginosis (N = 21,547; 23 studies; 13 countries). For syphilis, *N. gonorrhoeae,* and *C. trachomatis* infections, the pooled prevalence estimates for East and Southern Africa, as well as West and Central Africa, all fell between the lowest and highest point estimates for the sub-Saharan region presented in the 2005 review of curable STIs/RTIs by Mullick *et al.*[168] However, pooled prevalence estimates for *T. vaginalis* in both sub-regions were greater than highest point estimate reported by Mullick *et al.*, as was the pooled prevalence of bacterial vaginosis in East and Southern Africa compared to Mullick's highest point estimate. It is not possible to assess

whether this reflects a regional trend, or if the review by Mullick *et al.* understated the prevalence of *T. vaginalis* and bacterial vaginosis.

Since publication of the systematic review and meta-analysis, Davey *et al.* conducted a similar analysis for the period 2010 to 2015.[507] Their sub-regions were slightly different and 'Southern Africa' included South Africa, the only sub-Saharan country excluded from the analysis presented in this thesis because malaria is no longer endemic there. The authors stated, "Other systematic reviews of STI prevalence in pregnant women have found similarly high rates of STIs. A review article by Chico *et al.* in 2012[9] found that the prevalence of syphilis was 3.5%, CT was 6.1%, and TV was 17.8% amongst pregnant women in West and Central Africa."

The fact that only one study of the 171 identified in the systematic review of malaria and curable STIs/RTIs actually reported the incidence of co-infection is partially the result of researchers operating in scientific silos. Investigating co-infections that cut across disciplines requires researchers to become subject matter experts in more than one field, or to work in multi-disciplinary teams. Neither approach is easy, but either is necessary since IPTp-SP contributes to preventing adverse birth outcomes attributable to malaria infection and curable STIs/RTIs in pregnancy. Importantly, even if sulphadoxine is only partially protective against a broad-spectrum of gram-positive and gram-negative bacteria, dosing at each scheduled ANC visit from the second trimester to delivery may be sufficient to curb bacterial densities, thus directly reducing the incidence of adverse birth outcomes. It is possible, as well, that reduced

bacterial densities produced by sulphadoxine exposure also inhibit maternal inflammatory responses to infection that are known to trigger preterm birth.[508] However, can we do better?

In the case of curable STIs/RTIs, the ANC package recommended by the WHO includes screening for syphilis and the provision of BPG to women who test positive. Screening and treatment would need to continue even if dihydroartemisinin-piperaquine plus azithromycin were used because insufficient quantities of azithromycin perfuse the placenta to cure congenital cases. However, azithromycin would be curative of maternal syphilis, *N. gonorrhoeae*, *C. trachomatis*, and potentially *T. vaginalis.* Management of bacterial vaginosis in pregnancy may be more difficult. Treatment of women who have Nugent scores of 1-3 has not reduced the incidence of preterm birth. Based on evidence from this monograph, it would appropriate to conduct *in vitro* sensitivity testing of sulphadoxine with isolates from *T. pallidum, N. gonorrhoeae*, *C. trachomatis*, *T. vaginalis,* and organisms associated with bacterial vaginosis including *G. vaginalis, Bacteroides* spp*., Mobiluncus* spp. and *M. hominis.*

In light of the protection against curable STIs/RTIs conferred by IPTp-SP, it would be appropriate to conduct *in vitro* sensitivity testing of sulphadoxine versus azithromycin exposed to the five curable STIs/RTIs of interest and, potentially, other pathogens to begin to elucidate the range of protective effect pregnant women are benefitting from in countries where IPTp-SP is policy.

HIV-infected pregnant women also need an alternative to SP. At present, HIV-infected women are given cotrimoxazole against opportunistic infections; IPTp-SP is contra-indicated for these women out of concerns related to potential sulpha-toxicity if both therapies are used concomitantly. The combination of dihydroartemisinin-piperaquine plus azithromycin may represent a therapy that could be used alongside cotrimoxazole. Drug-drug interaction studies would need to confirm the safety of such use and, importantly, that maternal viral load is not adversely impacted. Thus, studies are now needed to determine whether dihydroartemisinin-piperaquine plus azithromycin would be a better alternative to SP for IPTp.

There are concerns that use of azithromycin may select for resistances in the pneumococcus. A single 1g dose of azithromycin clears ocular strains of *Chlamydia* that cause trachoma. During the past two decades, azithromycin has been distributed in trachoma-endemic areas as part of the WHO-led trachoma elimination programme.[509] Mass distribution has been shown also to reduce the incidence of respiratory infections,[510] diarrhoea,[511] and malaria.[512] These secondary benefits likely underlie the statistically significant reductions in all-cause childhood mortality reported in Ethiopia following trachoma treatment campaigns where odds were cut by one-half (OR = 0.51; 95% CI: 0.29-0.90) in a clinical trial[513] and 35% (OR = 0.35; 95% CI: 0.17–0.74) in a cohort study. However, there is significant evidence of clonal expansion of pneumococcal multi-locus sequence types *ermB* and *mefA/E* associated with resistance in the same geographic areas where four rounds of mass drug administration with azithromycin have been conducted.[514] Follow up surveillance

studies of six or more months post-exposure have found that the prevalence of resistant isolates do return to pre-dosing levels.[515,516] Importantly, there is no evidence of noticeable increases in pneumococcal resistance over time in countries that have participated in mass campaigns where over 500 million doses have been administered to date.[517] Regardless, to address this concern, trials that investigate the use of dihydroartemisinin-piperaquine plus azithromycin should test for macrolide resistance in the pneumococcus pre- and six or more months post-exposure. In addition, if dihydroartemisinin-piperaquine plus azithromycin were to be adopted for preventive care as part of the ANC package, then countries should collect biological samples on a semi-annual basis from pregnant women at sentinel sites with the objective of monitoring *in vivo* and *in vitro* sensitivity to curable STIs/RTIs as well as pneumococcal infections.

Descriptive epidemiological studies are needed to understand better the extent to which symptomatic versus asymptomatic curable STIs/RTIs contribute to adverse birth outcomes. Such studies would be incomplete if the prevalence of co-infections were not also considered. The apparent failure to reduce the incidence of adverse birth outcomes following treatment for one infection may be masked by the presence of co-infection(s) that will only be mitigated with the use of combination therapies and consideration of downstream outcomes.

Finally, discussion of the future of IPTp, with SP or an alternative, needs to be placed in the context of broader malaria elimination efforts. As malaria transmission intensity decreases in endemic areas, pregnant women will be exposed to fewer infective bites.

This may mean that primi- and secundigravidae will not develop the protective immunoglobulin G that is associated with maternal parity and the *var 2 CSA* gene. Consequently, future multigravidae may be at greater risk of adverse birth outcomes attributable to malaria than multigravidae of today, warranting close surveillance at national and district levels of malaria cases by gravidae.

# Conclusions

Evidence presented in this doctoral thesis suggests that IPTp-SP continues to provide valuable protection against the incidence of LBW amongst all gravidae until very low levels of malaria transmission and that this protective effect persists amongst primi- and secundigravidae in areas where one-half of malaria parasites carry the 'super resistant' A581G mutation. Amongst multigravidae, however, there is only evidence of continued protection against LBW in areas where $\leq$ 10.1% of malaria parasites express the A581G mutation. The actual threshold for multigravidae may be higher, but is unknown with data that are currently available.

There has long been speculation that sulphadoxine, the antibiotic component of SP, is protective against more pathogens than simply malaria infection. Evidence presented in this thesis supports the assertion that SP is, indeed, also protective against the adverse birth outcomes attributable to curable STIs/RTIs. This finding would encourage improved operational planning and coordination between National Malaria Control Programmes and Maternal and Reproductive Health Programmes, and provide greater impetus to scale up the antenatal coverage of IPTp-SP. Regardless, the dual burden of malaria infection and curable STIs/RTIs is a public health concern and continues to exact a high toll on pregnancies in sub-Saharan Arica. Use of dihydroartemisinin-piperaquine plus azithromycin as IPTp could be superior to IPTp-SP in safeguarding against adverse birth outcomes attributable to malaria and curable STIs/RTIs. Head-to-head clinical research between SP and dihydroartemisinin-piperaquine plus azithromycin with robust microbiology is required to chart the pathway forward.

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